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DAVID ALAN WARREN
Relationships of Blood and Brain Halocarbon Solvent Concentrations to Neurobehavioral Toxicity
(Under the direction of CHAM DALLAS AND JAMES BRUCKNER)

The extrapolation to humans of neurobehavioral toxicity data collected in experimental animals, in the absence of comparative pharmacokinetic analysis, can lead to erroneous conclusions. However, if the assumption is true that halocarbon concentrations in the central nervous system of one species are equally as toxic in another, the behavioral response of humans to various halocarbon exposures might be predicted from animal data where brain dose, or a suitable dose surrogate, is correlated with changes in behavior. At present, interspecies extrapolations of neurobehavioral toxicity data on the basis of brain dose are virtually non-existent, due to the paucity of data on halocarbon tissue kinetics, neurobehavioral toxicity, and their quantitative relationship.

Time-courses of blood and brain concentrations and neurobehavioral toxicity were determined for two halocarbon solvents in two rodent species. Based on these data, quantitative relationships, or the lack thereof, have been reported between the degree of neurobehavioral toxicity and internal measures of dose. In the case of orally-administered perchloroethylene (PCE), relationships between blood and brain concentrations and operant performance were not discernable, due in part to an acute adaptation of rats to PCE's response suppressing effect. For inhaled 1,1,1-trichloroethane (TRI), blood and brain concentrations were strongly correlated with the rate of operant responding in rats. Responding was slightly in excess of control rates at low concentrations, and decreased in a linear fashion as blood and brain concentrations increased. A robust biphasic response was seen in the locomotor activity of mice exposed to TRI by inhalation. Locomotor activity increased monophasically as solvent concentrations increased to a threshold concentration, above which activity declined and eventually fell below the control level.

The blood and brain concentration time-course data are currently of value for the validation and refinement of physiologically-based pharmacokinetic (PBPK) models for animals. The behavioral data sets are important additions to the toxicology literature in that they include the first studies of PCE's effect on operant responding and TRI's effect on the operant responding of rats, and a vast expansion of the concentration range used in the one existing study of TRI's effect on locomotor activity. However, the behavioral and pharmacokinetic data will be particularly valuable when used together to validate PBPK models that can predict the tissue pharmacokinetic patterns of halocarbons in experimental animals, and thus the magnitude of neurobehavioral toxicity expected. Validation of a PBPK model using this data could be very useful once the model is scaled up to humans, allowing insight into what exposure levels may or may not produce behavioral effects in humans.

INDEX WORDS: Halogenated Hydrocarbon, Perchloroethylene, 1,1,1-Trichloroethane, Methyl Chloroform, Operant Behavior, Locomotor Activity, Pharmacokinetics, Neurobehavioral Toxicity

RELATIONSHIPS OF BLOOD AND BRAIN HALOCARBON SOLVENT CONCENTRATIONS TO NEUROBEHAVIORAL TOXICITY

by

DAVID ALAN WARREN

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RELATIONSHIPS OF BLOOD AND BRAIN HALOCARBON SOLVENT CONCENTRATIONS TO NEUROBEHAVIORAL TOXICITY

by

DAVID ALAN WARREN

Approved:

Chem E. Dallas
Co-Major Professor

James V. Bruckner
Co-Major Professor

August 29, 1995
Date

Approved:

Gordhan L. Patel
Dean of the Graduate School

August 30, 1995
Date

TO NAN

Whose faith, goodness of heart, strength of character, and resolve were, and forever will be, a source of pride and sustenance.

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INTRODUCTION AND REVIEW OF LITERATURE

The short-chain, aliphatic, halogenated hydrocarbons or halocarbons (e.g., carbon tetrachloride, chloroform, perchloroethylene, 1,1,1-trichloroethane, trichloroethylene) belong to the structurally diverse group of chemicals known as volatile organic compounds (VOCs). Large volumes of halocarbons are used in industry as dissolvents, metal degreasers, dry cleaning agents and chemical intermediates. Consumer products such as cleaning fluids, paints and glues also frequently contain halocarbons. The numerous uses for these high-volume chemicals, commonly referred to as solvents, provide ample opportunity for exposures to dangerously high concentrations during accidents or misuse in industry (Moser and Balster, 1985). In addition, in what is a major public health problem, solvents are intentionally inhaled as a form of recreational drug abuse (Evans and Balster, 1991). Low-level, chronic exposures to solvents are commonplace due to the widespread contamination of ambient air and drinking water supplies from industrial emissions, leaking underground storage tanks and chemical waste dumps (ATSDR, 1992; 1994).

The volatility and lipophilicity of solvents are distinguishing characteristics that make them prone to inhalation and unique in terms of their pharmacokinetics. Once solvent vapors enter the lungs, their lipid solubility allows them to traverse lipoprotein cell membranes and enter the blood with ease, often within seconds of exposure (Astrand *et al.*, 1973; Dallas *et al.*, 1994). Respiratory uptake varies between individual solvents in relation to their blood:air partition coefficients, which are determined by alveolocapillary membrane permeability and blood solubility (Baker *et al.*, 1985). Once absorbed, solvents are transported throughout the body by the circulatory system and

distributed among the various body tissues at a rate controlled by cardiac output and tissue blood flow (Andrews and Snyder, 1991). The greater the blood flow to a tissue, the faster is the delivery of the solvent, and the more rapidly will its concentration rise in that area (Kennedy and Longnecker, 1990). The rate at which a solvent leaves the blood to enter the organs is also a function of its blood:air partition coefficient. Solvents having a high blood:air partition coefficient leave the blood and enter the organs at a slow rate, whereas agents that have a low blood:air partition coefficient distribute more rapidly (Andrews and Snyder, 1991). The amount of solvent taken up by various body tissues depends on its affinity for each tissue, which increases in proportion to organ lipid content. As the tissues take up solvent, the partial pressure of the solvent vapor in tissues increases toward that of the arterial blood. Since the rate at which vapor diffuses from arterial blood to tissues varies with the partial pressure difference between them, tissue concentrations change rapidly in the early minutes of exposure with tissue uptake slowing as exposure continues (Kennedy and Longnecker, 1990). The main routes by which the body eliminates solvents are urinary and pulmonary excretion. Generally, a large percentage of absorbed solvent is enzymatically transformed into metabolites with polar functions and finally excreted into the urine. Other solvents undergo minimal metabolism and are excreted via the lungs as unchanged compound. These same pharmacokinetic features are also generally applicable to the oral and dermal exposure routes.

Halocarbon solvents are of particular concern to toxicologists because several are metabolized to highly reactive intermediates that are cytotoxic to liver and kidney cells and carcinogenic in laboratory animals (Andrews and Snyder, 1991; Williams and Weisburger, 1991). Understandably, therefore, a large amount of resources are expended on halocarbon research to define human cancer risk. The regulatory community has recently begun, however, to give more consideration to regulating these chemicals on the basis of non-cancer health endpoints such as reproductive, respiratory

and neurological effects (U.S. EPA, 1988). The neurological endpoint is especially interesting because halocarbons easily cross the blood-brain barrier and gain access to nervous system tissue, which acts as a repository by virtue of its high blood perfusion and high lipid content (Baker and Fine, 1986). While the occurrence of high solvent concentrations in the central nervous system (CNS) does not mean that it should be automatically regarded as target tissue, virtually all solvents are capable of producing an acute general depression of the CNS. Indeed, several of the halocarbons were at one time employed as inhalational anesthetics because of their acute narcotic effects (Baker *et al.*, 1985). There is also increasing evidence that long-term, relatively low-level exposures to halocarbons can result in chronic toxic encephalopathy characterized by impaired intellect, memory and personality (Arlien-Soborg, 1992). While investigations of chronically exposed laboratory animals are desperately needed, this dissertation focuses on the acute effects of relatively high-level solvent exposures.

Regardless of the halocarbon, the results of acute high-level exposure are quite similar. Irritant effects are seen initially in the form of eye, nose and throat irritation, mild headache and nausea. With continued exposure, sleepiness, fatigue, difficulty in concentration, personality and mood changes, blunted judgement and impaired reaction speed characterize the human syndrome. With higher exposure levels or prolonged durations of exposure, progression to ataxia, unconsciousness, and death from respiratory or cardiovascular arrest is typically observed. This toxicity profile, consisting mostly of CNS effects, is similar to that produced by sedative-hypnotic drugs and other VOCs such as ethanol and gaseous anesthetics (Evans and Balster, 1991). As for the two halocarbons employed in this dissertation, perchloroethylene (PCE) has produced mood changes, memory loss, insomnia, incoordination, and perceptual, attention and intellectual deficits in humans (Lauwerys *et al.*, 1983; Gregersen, 1988; Seeber, 1989). Acute exposures to 1,1,1-trichloroethane (TRI) have impaired performance in tests of manual dexterity, eye-hand coordination, perceptual speed and reaction time, and

produced less subtle effects such as lightheadedness and imbalance (Torkelson *et al.*, 1958; Stewart *et al.*, 1961; Gamberale and Hultengren, 1973; Mackay *et al.*, 1987).

It is widely accepted that the halocarbons themselves, as opposed to metabolites, are responsible for acute CNS effects (Andrews and Snyder, 1991). This is supported by the rapidity with which symptoms develop and the effectiveness of halocarbons that are metabolized to a very limited degree. Further evidence for the role of parent compound is the clear linear relationship between the anesthetic potency and blood:air partition coefficients of halocarbons (Sato and Nakajima, 1987). In addition, the similarity of symptoms produced by solvents suggests that the effects result from a common mechanism unlikely to result from a heterogeneous collection of metabolites.

There remains no satisfactory explanation as to how halocarbons produce their acute CNS effects, but these agents are thought to generalize to ethanol and the gaseous anesthetics regarding mechanism of action. Once absorbed, they distribute to sites in which they are removed from the aqueous environment. For this reason, they are believed to exert their effects by dissolving in lipid membranes to cause a local disordering of the lipid matrix, thereby perturbing the function of ion channels and proteins embedded therein (Rall, 1990). This hypothesis is strongly supported by the excellent correlation between lipid solubility and potency of gaseous anesthetics (Kennedy and Longnecker, 1990). Moreover, the hydrophobic domains of membrane-bound proteins appear to represent additional targets for anesthetic agents. In this regard, attention has been focused on the capacity of ethanol and aliphatic alcohols to augment GABA-mediated synaptic inhibition as well as fluxes of chloride (Rall, 1990). Ethanol has also been shown to block the N-methyl-D-aspartate receptor in brain cells and to inhibit the related production of cyclic GMP at levels in the range that would be expected to produce mild intoxication (Andrews and Snyder, 1991). Additionally, solvents have been shown to initiate the stress response by prompting the release of corticotropin releasing hormone in the CNS (Glowa, 1990). Therefore, solvents may exert many

physiological effects in the CNS, some of which may have functional or behavioral consequences.

Despite recommendations by several National Academy of Science (NAS) committees that examinations of learned and unlearned animal behaviors (as well as morphology) constitute the first steps in chemical hazard identification and evaluation (Buckholtz and Panem, 1986), there is no requirement that all chemicals be tested for neurological effects before being introduced into commerce. In September 1993, however, the U.S. Environmental Protection Agency (EPA) for the first time began requiring manufacturers to measure the neurobehavioral toxicity of ten specific solvents using cognitive function and screening level tests, including schedule-controlled operant behavior (SCOB) and locomotor activity, the two tests employed in this dissertation (U.S. EPA, 1993). The recommendations of the National Academy of Sciences and EPA's multi-solvent test rule are significant since toxicity has traditionally been defined by tissue damage or by death, and functional changes, of which behavioral changes represent one type, have been considered only cursorily (Sette and Levine, 1986). An increased emphasis on neurobehavioral toxicity testing seems justified since behavior represents a functional integration of the nervous system and, therefore, nervous system capacity cannot be determined in histological or even physiological studies independent of behavioral analyses (Tilson and Mitchell, 1984). To the majority of toxicologists, however, subjective complaints and slight reductions in performance remain trivial endpoints, and the acute effects of solvents are too vague to engage their attention. In certain settings, however, the implications of such effects can be overwhelming. Consider the potential significance of a slight rise in reaction time to a fighter pilot (Weiss, 1988), sedation to an operator of heavy machinery, and difficulties in concentration to a glue-sniffing high school student. Such solvent effects are generally considered reversible and of little or no pathological consequence (Winneke, 1982), and are the subject matter of behavioral toxicology.

The field of behavioral toxicology is in its infancy. It was 1969 before Weiss and Laties authored the first formal discussion of behavioral toxicology in the *Annual Review of Pharmacology* (Sette and Levine, 1986). Three years later, in what is also considered a seminal event, the Fifth Rochester Conference on Environmental Toxicity was devoted to behavioral toxicology (Sette and Levine, 1986). Despite its brief history, the science of neurobehavioral toxicity testing is well developed due to its use of techniques that have proven reliable through decades of psychopharmacology research with drugs. In addition to reliability, surveys of known neurotoxicants clearly indicate that similar neurological and behavioral signs are produced in humans and experimental animals, indicating that behavioral testing has predictive validity (Spencer and Schaumberg, 1980). Behavioral tests in animals have also proven to be sensitive measures of toxicant exposure, as many agents elicit functional deficits prior to the onset of more obvious toxic effects or morphological damage (Tilson and Mitchell, 1984).

Although neither has been applied extensively to industrial solvents, two of the most popular behavioral measures are SCOB and locomotor activity. Changes in these measures are thought to reflect effects of solvents in laboratory animals comparable to psychomotor changes in humans (ATSDR, 1994). Data collection is done with automated equipment in both cases, thereby diminishing investigator bias. Both SCOB and locomotor activity also result in continuous quantitative data that are sufficient to reflect the dynamics of solvent uptake and distribution. Locomotor activity has the added advantage of being relatively rapid, in contrast to SCOB where animal training is often time-consuming and labor intensive. Schedule-controlled operant behavior and locomotor activity are, however, of limited use in determining mechanisms or sites of action since they are susceptible to change by alterations in several neurobehavioral processes (i.e., sensory, motor, motivational, associative) (Rafales, 1986). This is compounded for solvents, which are ubiquitously distributed within the brain (Gospe and Calaban, 1988; Ameno *et al.*, 1992).

Studies of toxicant effects on SCOB typically establish a dependency between the occurrence of a specific response (e.g., lever-press) and the intermittent presentation of a specific stimulus (e.g., milk). Eventually the lever-pressing of the subject, usually a mouse or rat, is reliably maintained by the reinforcing stimulus resulting in stable rates of lever-pressing over time. Once steady-state responding is established, changes in the rate of responding are usually examined as a function of exposure concentration or administered dose. Studies employing SCOB have demonstrated behavioral effects for a number of solvents including toluene, trichloroethylene and TRI (Dews, 1978; Glowa *et al.*, 1983; Wood *et al.*, 1983; Moser and Balster, 1986). Excellent general introductions to the use of SCOB in behavioral toxicology have been written by Laties and Wood (1986) and Rice (1988).

Like SCOB, locomotor activity represents some interactive outcome of information processing, and therefore reflects the functional state of the nervous system. Narrowly defined, it is horizontally directed movement using the limbs, and is thus distinct from general motor activity which also includes vertically directed movement, sniffing and grooming. Locomotor activity measures are typically made in rodents and reflect the frequency of movement or spatial displacement of the animal per unit of time. Changes in the frequency of motor activity have been documented for PCE, TRI, trichloroethylene and toluene (Kjellstrand *et al.*, 1985; Wood and Colotla, 1990). The assessment of locomotor activity has been reviewed by Rafales (1986).

To date, the field of behavioral toxicology has focused on the identification of agents with toxic effects and the characterization of those effects in laboratory animals. As alluded to earlier, an expanded role for neurobehavioral toxicology as a tool for regulatory decision making is foreseen. Since regulatory decisions are based in part on chemical risk assessments, which in turn are largely based on dose-response curves, this expansion would depend upon the availability of behavioral dose-response data. Unfortunately, few studies have generated dose-response data for solvent effects on

animal behavior. For example, although TRI is the best characterized behaviorally of all the halocarbons, only three studies of the dose-dependency of its effects on steady-state operant responding have been reported (Balster *et al.*, 1982; Moser *et al.*, 1985; Moser and Balster, 1986). No studies of PCE's effect on operant behavior have been conducted. In addition, only one published study was located that examined the locomotor activity effects of TRI and PCE at more than one dose (Kjellstrand *et al.*, 1985).

Dose-response relationships in experimental animals would be particularly valuable if animals reacted similarly to humans upon inhaling (or ingesting) the same concentration of solvent. Unfortunately, the assumption that they do so cannot be made since physiological differences exist among species that ultimately influence the amount and time-course of solvent deposition in the brain. The extrapolation of behavioral dose-response data generated in experimental animals, in the absence of comparative pharmacokinetic analysis, may therefore overstate or underestimate human risk. As a result, it has been suggested that a scientifically-defensible approach to making interspecies extrapolations would assume that a particular target tissue dose in one species is equally as toxic in another (Andersen, 1987). Such an approach would benefit greatly from dose-response relationships where brain dose (i.e., brain concentration) or a suitable dose surrogate is correlated with behavioral changes. Brain concentration would appear to be a reasonable choice for correlation purposes, since solvent effects on behavior are thought to reflect the consequences of neuronal membrane fluidization, the degree of which is proportional to the amount of solvent dissolved therein. Studies by Bruckner and Peterson (1981) and Ameno *et al.* (1992) suggest that the solvent concentration in blood may be a suitable surrogate for brain concentration, provided sufficient time has elapsed for blood and brain concentrations to equilibrate.

Excluding studies relating alcohol-induced effects and blood ethanol levels (Sidell and Pless, 1971; Jones and Vega, 1972; Radlow and Hurst, 1985), only two animal studies

could be located in which solvent effects on the CNS were related to internal measures of dose. Bruckner and Peterson (1981) have shown that the magnitude of toluene-induced impairment of reflexes and unconditioned performance in mice is paralleled by blood and brain concentrations of toluene. More recently, Kishi *et al.* (1983) have reported a relationship between blood trichloroethylene levels and shock avoidance performance decrements in rats. It is clear that due to ethical considerations, further progress in understanding the risk that solvents pose to the human CNS rests with performing animal studies. It is therefore imperative that sensitive behavioral tests be used in conjunction with physiologically and anatomically well characterized animals to create a data base to which state-of-the-art extrapolation methods can be applied. *Therefore, the primary goal of this dissertation is to quantitatively relate blood and brain halocarbon solvent concentrations to the degree of neurobehavioral toxicity in rats and mice, as measured by changes in schedule-controlled operant responding and locomotor activity.*

In view of the large number of solvents in use today and the continual introduction of new agents, rapid and inexpensive methods will be needed to assess their behavioral toxicity and its relationship to dose. Due primarily to the laborious task of training animals, the practicality of SCOB for dose-response determinations depends largely on the repeated administration of different doses of the test substance to the same experimental animal. In turn, the appropriateness of such a within-subject design depends upon whether or not chemical exposure irreversibly changes an animal's behavior in such a manner that would be reflected in its behavioral reaction to subsequent exposures (Sidman, 1960).

The generation of cumulative dose-response curves by exposing animals to incrementally increasing solvent concentrations in a single operant session is a widely accepted practice in behavioral toxicology (Glowa *et al.*, 1983; Glowa, 1991; Glowa, 1993). With such a design, the potential for solvent accumulation is great, and exposure

to early solvent concentrations might be expected to influence the behavioral reaction to subsequent exposures. Because solvents classically exhibit a rapid rate of elimination, an alternative means of generating dose-response curves using a within-subject design would be to allow sufficient time between exposures for complete solvent clearance. The question remains, however, whether or not solvent exposure in the absence of solvent accumulation, would also influence an animal's response to subsequent exposures. While evidence suggest that a residual effect of exposure may occur for carbon disulphide and toluene (Glowa, 1981; Liang *et al.*, 1983), evidence also suggest that this is not a generalized phenomenon (Glowa *et al.*, 1983; Glowa and Dews, 1987).

If a residual effect does exists, repeated exposure of animals to solvents may not result in dose-response curves comparable to those generated using chemically-naive subjects. Thus, comparisons of single and repeated exposures are essential for fully accurate interpretations of the behavioral consequences of solvent exposure. *Therefore, a second goal of this dissertation is to compare dose-response curves for the effects of TRI on the operant behavior of singly and repeatedly exposed rats, in order to determine whether or not the behavioral effects of rate-decreasing concentrations are augmented by previous exposures.*

REFERENCES

Ameno, K., Kiriu, T., Fuke, C., Ameno, S., Shinohara, T., and Ijiri, I. (1992). Regional brain distribution of toluene in rats and in human autopsy. *Archives of Toxicology*. 66, 153-156.

Andersen, M.E. (1987). "Tissue dosimetry in risk assessment, or what's the problem here anyway?" In: *Drinking Water and Health*, Vol.8, pp. 8-26. National Academy Press, Washington, DC.

Andrews, L.S., and Snyder, R. (1991). Toxic effects of solvents and vapors. In: *Casarett and Doull's Toxicology-The Basic Science of Poisons*. Fourth Ed., (Eds. Amdur, M.O., Doull, J., and Klaassen, C.D.). Pergamon Press, NY. pp. 681-722.

Arlien-Soborg, P. (1992). *Solvent Neurotoxicity*. CRC Press, Inc., Boca Raton, FL., 1-10.

Astrand, L., Kilbom, A., Wahlberg, I., and Ovrum, P. (1973). Methylchloroform exposure. I. Concentration in alveolar air and blood at rest and during exercise. *Work Environ. Health.* 10, 69-81.

(ATSDR) Agency for Toxic Substances and Disease Registry. (1992). *Toxicological Profile for Tetrachloroethylene.* TP-92/18. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

(ATSDR) Agency for Toxic Substances and Disease Registry. (1994). *Toxicological Profile for 1,1,1-Trichloroethane.* U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Baker, E.L., and Fine, L.J. (1986). Solvent neurotoxicity: the current evidence. *J. Occup. Med.* 28(2), 126-129.

Baker, E.L., Smith, T.J., and Landrigan, P.J. (1985). The neurotoxicity of industrial solvents: A review of the literature. *Am. J. Ind. Med.* 8, 207-217.

Balster, R.L., Moser, V.C., and Woolverton, W.L. (1982). Concurrent measurements of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J. Pharmacol. Methods*. 8, 299-309.

Bruckner, J.V., and Peterson, R.G. (1981). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol. Appl. Pharmacol.* 61, 27-38.

Buckholtz, N.S., and Panem, S. (1986). Regulation and evolving science: Neurobehavioral toxicology. *Neurobeh. Toxicol. Teratol.* 8, 89-96.

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. (1994). Development of a physiologically based pharmacokinetic model for perchloroethylene using tissue concentration-time data. *Toxicol. Appl. Pharmacol.* 128, 50-59.

Dews, P.B. (1978). Epistemology of screening for behavioral toxicity. *Environ. Health Perspect.* 26, 37-42.

Evans, E.B., and Balster, R.L. (1991). CNS depressant effects of volatile organic solvents. *Neuroscience and Biobehavioral Reviews.* 15, 233-241.

Gamberale, F., and Hultengren, M. (1973). Methylchloroform exposure II. Psychophysiological functions. *Scand. J. Work Environ. Health.* 10, 82-92.

Glowa, J.R. (1981). Some effects of sub-acute exposure to toluene on schedule-controlled behavior. *Neurobehav. Toxicol. Teratol.* 3, 463-465.

Glowa, J.R. (1990). Behavioral toxicology of solvents. *Drug Development Research.* 20, 411-428.

Glowa, J.R. (1991). Behavioral toxicology of volatile organic solvents V. Comparisons of the behavioral and neuroendocrine effects among n-Alkanes. *J. Am. Coll. Toxicol.* 10(6), 639-646.

Glowa, J.R. (1993). Behavioral and neuroendocrine effects of diethyl ether exposure in the mouse. *Neurotoxicol. Teratol.* 15, 215-221.

Glowa, J.R., and Dews, P.B. (1987). Behavioral toxicology of volatile organic solvents. IV. Comparisons of the rate-decreasing effects of acetone, ethyl acetate, methyl ethyl ketone, toluene, and carbon disulphide on schedule-controlled behavior of mice. *J. Am. Coll. Toxicol.* 6(4), 461-469.

Glowa, J.R., DeWeese, M.E., Natale, M.E., Holland, J.J., and Dews, P.B. (1983). Behavioral toxicology of volatile organic solvents. I. Methods: acute effects. *J. Am. Coll. Toxicol.* 2, 175-185.

Gospe, S.M., and Calaban, M.J. (1988). Central nervous system distribution of inhaled toluene. *Fund. Appl. Toxicol.* 11, 540-545.

Gregersen, P. (1988). Neurotoxic effects of organic solvents in exposed workers: Two controlled follow-up studies after 5.5 and 10.6 years. *Am. J. Ind. Med.* 14, 681-701.

Jones, B.M., and Vega, A. (1972). Cognitive performance measured on the ascending and descending limb of the blood alcohol curve. *Psychopharmacologia.* 23, 99-114.

Kennedy, S.K., and Longnecker, D.E. (1990). History and principles of anesthesiology. In: *Goodman and Gilman's, The Pharmacological Basis of Therapeutics*. Eighth Ed. (Eds. Goodman Gilman, A., Rall, T.W., Nies, A.S., and Taylor, P.). Pergamon Press, NY. pp. 269-284.

Kishi, R., Harabuchi, I., Ikeda, T., Katakura, Y., and Miyake, H. (1993). Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans. *Br. J. Ind. Med.* 50, 470-480.

Kjellstrand, P., Holmquist, B., Jonsson, I., Romare, S., and Mansson, L. (1985). Effects of organic solvents on motor activity in mice. *Toxicology*. 35, 35-46.

Laties, V.G., and Wood, R.W. (1986). Schedule-controlled behavior in behavioral toxicology. In: *Neurobehavioral Toxicology*. (Ed. Annau, Z.). The Johns Hopkins University Press, 69-93.

Lauwerys, R., Herbrand, J., Buchet, I.P., Bernard, A., and Graussin, J. (1983). Health Surveillance of workers exposed to tetrachloroethylene in dry-cleaning shops. *Int. Arch. Occup. Environ. Health.* 52, 69-77.

Liang, Y-X, Glowa, J.R., and Dews, P.B. (1983). Behavioral toxicology of volatile organic solvents. III. Acute and subacute effects of carbon disulphide exposure on the behavior of mice. *J. Am. Coll. Toxicol.* 2(6), 379-389.

Mackay, C.J., Campbell, L., Samuel, A.M., Alderman, K.J., Idzikowski, C., Wilson, H.K., and Gompertz, D. (1987). Behavioral changes during exposure to 1,1,1-trichloroethane: Time-course and relationship to blood solvent levels. *Am. J. Ind. Med.* 11, 223-239.

Moser, V.C., and Balster, R.L. (1985). Acute motor and lethal effects of inhaled toluene, 1,1,1-trichloroethane, halothane and ethanol in mice: Effects of exposure duration. *Toxicol. Appl. Pharmacol.* 77, 285-291.

Moser, V.C., and Balster, R.L. (1986). The effects of inhaled toluene, halothane, 1,1,1-trichloroethane, and ethanol on fixed-interval responding in mice. *Neurobehav. Toxicol. Teratol.* 8, 525-531.

Moser, V.C., Scimeca, J.A., and Balster, R.L. (1985). Minimal tolerance to the effects of 1,1,1-trichloroethane on fixed-ratio responding in mice. *Neurotoxicology*. 6(1), 35-42.

Radlow, R., and Hurst, P.M. (1985). Temporal relations between blood alcohol concentration and alcohol effect: an experiment with human subjects. *Psychopharmacology*. 85, 260-266.

Rafales, L.S. (1986). Assessment of Locomotor Activity. In: *Neurobehavioral Toxicology*. (Ed. Annau, Z.). The Johns Hopkins University Press, 54-68.

Rall, T.W. (1990). Hypnotics and sedatives; ethanol. In: *Goodman and Gilman's, The Pharmacological Basis of Therapeutics*. Eighth Ed. (Eds. Goodman Gilman, A., Rall, T.W., Nies, A.S., and Taylor, P.). Pergamon Press, NY. pp. 2345-382.

Rice, D.C. (1988). Quantification of operant behavior. *Toxicology Letters*. 43, 361-379.

Sato, A., and Nakajima, T. (1987). Pharmacokinetics of organic solvent vapors in relation to their toxicity. *Scand. J. Work Environ. Health*. 13, 81-93.

Seeber, A. (1989). Neurobehavioral toxicity of long-term exposure to tetrachloroethylene. *Neurotoxicol. Teratol.* 11, 579-583.

Sette, W.F., and Levine, T.F. (1986). Behavior as a regulatory endpoint. In: *Neurobehavioral Toxicology*. (Ed. Annau, Z.). The Johns Hopkins University Press, 391-403.

Sidell, F.R., and Pless, J.E. (1971). Ethyl alcohol: Blood levels and performance decrements after oral administration to man. *Psychopharmacologia.* 19, 246-261.

Sidman, M. (1960). *Tactics of Scientific Research*. Authors Cooperative, Inc., Boston, MA., 42-67.

Spencer, P.S., and Schaumberg, H.H. (1980). *Experimental and Clinical Neurotoxicolgy*. Baltimore: Williams and Wilkins.

Stewart, R.D., Gay, H.H., Erley, D.S., Hake, C.L., and Schaffer, A.W. (1961). Human exposure to 1,1,1-trichloroethane vapour: relationship of expired air and blood concentrations. *Am. Ind. Hyg. Assoc. J.* 22, 252-262.

Tilson, H.A., and Mitchell, C.L. (1984). Neurobehavioral techniques to assess the effects of chemicals on the nervous system. *Ann. Rev. Pharmacol. Toxicol.* 24, 425-450.

Torkelson, T.R., Oyen, F., McCollister, D.D., and Rowe, V.K. (1958). Toxicity of 1,1,1-trichloroethane as determined on laboratory animals and human subjects. *Am. Ind. Hyg. Assoc. J.* 19, 353-362.

U.S. EPA (United States Environmental Protection Agency) (1993). "Multi-substance rule for the testing of neurotoxicity." Section 4(a), Toxic Substances Control Act.

U.S. EPA (United States Environmental Protection Agency) (1988). "Research to improve health risk assessments (RIHRA) program." Office of Research and Development. Washington, DC. EPA/600/8-88/089. pp. 1-14.

Weiss, B. (1988). Quantitative perspectives on behavioral toxicology. *Toxicology Letters.* 43, 285-293.

Williams, G.M., and Weisburger, J.H. (1991). Chemical Carcinogenesis. In: *Casarett and Doull's Toxicology-The Basic Science of Poisons.* Fourth Ed., (Eds. Amdur, M.O., Doull, J., and Klaassen, C.D.). Pergamon Press, NY. pp. 127-200.

Winneke, G. (1982). Acute behavioral effects of exposure to some organic solvents-psychophysiological aspects. *Acta. Neurol. Scandinav.*, Suppl.92, Vol.66, 117-129.

Wood, R.W., and Colotla, V.A. (1990). Biphasic changes in mouse motor activity during exposure to toluene. *Fundamental and Applied Toxicology.* 14, 6-14.

Wood, R.W., Rees, D.C., and Laties, V.G. (1983). Behavioral effects of toluene are modulated by stimulus control. *Toxicol. Appl. Pharmacol.* 68, 462-472.

SCHEDULE-CONTROLLED OPERANT BEHAVIOR OF RATS FOLLOWING ORAL ADMINISTRATION OF PERCHLOROETHYLENE: TIME-COURSE AND RELATIONSHIP TO BLOOD AND BRAIN SOLVENT LEVELS[1]

[1]Warren, D.A., Reigle, T.G., Muralidhara, S., and Dallas, C.E. Accepted by the *Journal of Toxicology and Environmental Health*, 6/7/95.

ABSTRACT

Previous studies have indicated that human exposure to perchloroethylene (PCE) produces subtle behavioral changes and other neurological effects at concentrations at or below the current occupational exposure limit. Since comparable effects in animals may be reflected by changes in schedule-controlled operant behavior, the ability of orally administered PCE to alter fixed-ratio (FR) responding for a food reward was investigated in male Sprague-Dawley rats. Furthermore, since behavioral effects of solvents are likely to be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose, the relationship between the pharmacokinetic distribution of PCE and its effects on operant responding was also evaluated. Rats trained to lever-press for evaporated milk on a FR-40 reinforcement schedule were gavaged with 160 or 480 mg/kg PCE and immediately placed in an operant test cage for 90 minutes. Separate animals gavaged with equivalent doses of PCE were used to determine profiles of blood and brain concentrations versus time. Perchloroethylene produced changes in responding that not only varied with dose, but also among animals receiving the same dose. Changes in the response rates of rats receiving 160 mg/kg were either not readily apparent, restricted to the first 5 minutes of the operant session or attributable to gavage stress and the dosing vehicle. However, 480 mg/kg produced either an immediate suppression of responding for 15-30 minutes before a rapid recovery to control rates or a complete elimination of lever-pressing for the majority of the operant session. Although the two doses of PCE produced markedly different effects on operant behavior during the first 30 minutes of exposure, differences in brain concentrations of PCE were minimal. Furthermore, the majority of animals receiving 480 mg/kg fully recovered from response suppression while blood and brain levels of the solvent continued to rise. Thus, relationships between blood and brain PCE levels and performance impairment were not discernable over the monitored time-course. Since the rapid onset of response

suppression suggests that the precipitating event occurs within the first few minutes of exposure, it is possible that altered responding is related to the rate of increase in blood or brain concentrations rather than the absolute solvent concentrations themselves. The relationship between the pharmacokinetic distribution of solvents and their effects on the central nervous system is obviously complex and may involve acute neuronal adaptation as well as the dynamics of solvent distribution among the various body compartments.

INTRODUCTION

Perchloroethylene (1,1,2,2-tetrachloroethylene, PCE) is a volatile organic solvent which is used in large quantities as a dry cleaning agent, chemical intermediate and metal degreaser (ATSDR, 1993). Approximately 650,000 workers in the United States are estimated to be at risk for occupational exposure to PCE (NOES, 1990). Exposure surveys of the dry cleaning industry have determined that mean time-weighted average exposures typically range from 28.2-88.2 ppm (Materna, 1985) and 4-149 ppm (Ludwig *et al.*, 1983). Much higher exposures are associated with cleaning spills or replacing dry-cleaning filters (ATSDR, 1990). The 8-hour, time-weighted average workplace exposure limit for PCE is 100 ppm.

There is evidence that PCE is a rodent carcinogen (NCI, 1977; NTP, 1986), but such evidence in humans is equivocal. While PCE is a hepatic and renal toxicant at high doses, the majority of reports of human toxicity has focused upon neurological effects among those occupationally (Gregersen, 1988; Ferroni *et al.*, 1992) or experimentally (Rowe *et al.*, 1952; Stewart *et al.*, 1970) exposed by inhalation. In these studies, acute exposures in the 100-200 ppm range were found to produce reversible mood changes and impaired coordination. Major EEG changes suggestive of cerebral cortical depression have also been found among volunteers repeatedly exposed to 100 ppm (Hake and Stewart, 1977). Subchronic exposure to even lower PCE concentrations has reportedly

caused memory loss and insomnia (Lauwerys *et al.*, 1983), as well as perceptual, attention and intellectual deficits (Seeber, 1989). Such studies suggest that PCE may have neurological effects at or below the current occupational exposure limit, some of which may be manifest as subtle behavioral changes.

While the ability of PCE to increase the locomotor activity of laboratory animals has been reported (Savolainen *et al.*, 1977; Kjellstrand *et al.*, 1985; Fredriksson *et al.*, 1993), PCE's effect on schedule-controlled operant behavior (SCOB) has not been investigated. Studies employing SCOB have, however, demonstrated behavioral effects for a number of other solvents, including toluene (Glowa *et al.*, 1983; Wood *et al.*, 1983), 1,1,2-trichloroethylene (Dews, 1978; Kulig, 1987) and 1,1,1-trichloroethane (Balster *et al.*, 1982; Moser and Balster, 1986). Because the primary route of exposure to volatile solvents is inhalation, it was the original intent of this investigation to concurrently measure various PCE vapor concentrations and effects on SCOB. Attempts at doing so were unsuccessful, however, since rats trained to lever-press for evaporated milk immediately ceased responding upon exposure to even modest concentrations of PCE vapor. Case studies of an accidental poisoning victim (Koppel *et al.*, 1985) and patients administered PCE as an anthelmintic agent (Wright *et al.*, 1937; Sandground, 1941; Haerer and Udelman, 1964) have indicated that the acute neurological effects of PCE ingestion parallel those seen after inhalation. Therefore, SCOB was monitored after the oral administration of PCE. Because the behavioral effects of solvents may be more closely related to blood or target tissue (i.e., brain) concentrations than administered dose, the present study was designed to evaluate the relationship between the pharmacokinetic distribution of orally administered PCE and the effects of this agent on the SCOB of rats.

MATERIALS AND METHODS

Test Chemicals: Perchloroethylene of 99% + purity was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Burdick and Jackson Brand, High Purity Solvent isooctane was obtained from Baxter Healthcare Corp. (Muskegon, MI). Alkamuls® EL-620 (formerly Emulphor® EL-620), a polyethoxylated vegetable oil, was a gift of Rhone-Poulenc (Cranbury, NJ).

Animals: Male Sprague-Dawley (SD) rats (Charles River Breeding Laboratories, Raleigh, NC) weighing 300-350 g were used in all experiments. Rats were housed two per cage in suspended wire bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hr light-dark cycle (light: 0700-1900 hr). Rats were allowed to acclimate for a minimum of 7 days prior to use, during which time food (Purina Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*. All experiments were conducted during the light phase of the light-dark cycle.

Behavioral Apparatus: Operant sessions took place in a test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during availability of the milk reinforcer. The test cage was placed inside a 1.0 M^3 Rochester-type dynamic flow inhalation chamber that served to isolate animals from extraneous stimuli. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant schedule and recorded the number of responses and reinforcers in each 5-minute interval of the operant session.

Operant Behavior: Twelve rats (six per dose) were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire

lids. Rats were food-restricted during a period in which they were trained to lever-press for undiluted evaporated milk (0.05 ml for 7 seconds/reinforcement) on a fixed-ratio (FR) 40 reinforcement schedule (every 40th lever-press produced milk). Initially, rats were manually reinforced in 30-minute sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the ratio of responses to reinforcers was gradually increased to forty and the session length extended to 90 minutes. Rats were allowed to respond in daily 90-minute sessions spaced 24 hours apart until their response rates stabilized, a process requiring 15-20 days. The criterion for stable behavior was four successive sessions in which the number of responses/second varied by less than 15% from the 4-day mean rate. Upon the completion of each operant session, rats were returned to their home cages and given 10 $\pm$ 0.25 g of food which was promptly eaten. Once rats exhibited stable behavior, they were gavaged with either a 10 or 20% aqueous Alkamuls® vehicle (3 ml/kg), immediately placed in a modular test cage and monitored for operant behavior for 90 minutes. Twenty-four hours later, rats were dosed with either 160 or 480 mg/kg PCE in the appropriate vehicle and their behavior monitored again. Ten (160 mg/kg) or 20% (480 mg/kg) Alkamuls® EL-620 was used to prepare stable emulsions of PCE in 0.9% saline. The actual concentration of PCE in each dosage formulation was confirmed by headspace gas chromatography.

Blood Sampling: Twelve rats (six per dose) were food restricted (10 $\pm$ 0.25 g/day) for 72 hours prior to being surgically implanted with an indwelling carotid artery cannula. Rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture of ketamine HCL (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in the proportion 3:2:1 (v:v:v). The cannulae exited the skin at the nape of the neck and were protected from manipulation by surgical tape. After an overnight recovery period during which food was withheld ($\approx$ 18 hr), either 160 or 480 mg/kg PCE was administered as an aqueous Alkamuls® emulsion by bolus gavage in a total volume of 3 ml/kg. Following dosing, blood samples were withdrawn from the arterial

cannulae of unrestrained and unanesthetized animals via a three-way stopcock and 1 ml syringe. Serial blood samples (2-75 μl, depending upon the anticipated blood concentration) were taken at various times during the 4 days that followed dosing. Blood samples were quickly transferred to 8 ml headspace vials, capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly. Some blood samples required dilution with ice-cold saline in order to be analyzed within the linear range of the electron capture detector (ECD) of a gas chromatograph (GC). As necessary, blood withdrawal was followed by a heparin flush to maintain cannula patency. Food was available *ad libitum* during blood sampling.

Tissue Sampling: For the determination of PCE concentrations in tissues, rats were food restricted (10 $\pm$ 0.25 g/day) for 72 hours, and subsequently fasted for 18 hours prior to being dosed as described for blood sampling. Groups of six rats were sacrificed by cervical dislocation followed by decapitation at 1, 6, 15, 20, 30, 40, 50, 60 and 90 minutes after dosing. Approximately 0.5-1.0 g samples of brain, liver, perirenal fat and skeletal muscle were excised within 2.5-3 minutes from each rat and immediately placed into chilled scintillation vials containing 2 ml of 0.9% saline and 8 ml of isooctane. Tissues were homogenized as quickly as possible (5-15 seconds) with an Ultra-Turrax® homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of PCE, after which the sample was vortex-mixed for 30 seconds. The homogenates were then centrifuged at 2500 x g for 10 minutes at 4°C in the capped scintillation vials. An aliquot of the isooctane layer (5-20 μl) was either transferred directly to a 20 ml headspace vial or first diluted with isooctane. The vials were capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly.

PCE Analysis: A Sigma Model 300 GC equipped with a HS6 headspace sampler and an ECD (Perkin-Elmer Co., Norwalk, CT) was used for the analysis of PCE in blood. Analyses were carried out using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating

conditions were: headspace sampler temperature, 80°C; injection port temperature, 200°C; column temperature, 110°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/minute. For PCE analysis of isooctane tissue extracts, a Perkin-Elmer Model 8500 GC with a HS-101 headspace autosampler and ECD was employed under the same conditions as those previously listed. Perchloroethylene concentrations were calculated from daily prepared standard curves and were corrected for the percent recovery characteristic of blood and tissue samples. The percent recovery and blood/tissue extraction procedures have been described by Chen *et al.* (1993). The limit of detection for PCE was approximately 1 ng in 20 ml of air.

Data Analysis: RSTRIP (Version 3.1, 1988) (MicroMath, Inc., Salt Lake City, UT) was used to fit PCE blood concentration versus time profiles to polyexponential equations for the calculation of area under the blood concentration versus time curve (AUC), elimination half-life ($t_{1/2}$) and maximum blood concentration (C_{max}). Tests for differences in the concentrations of PCE in blood and tissues were made with two sample t-tests (T-ease Program, Version 2.0, Institute for Scientific Information, 1987). The control behavioral response of each rat was calculated as the mean number of lever-presses in each 5-minute interval of the four operant sessions used to meet the stability criterion. Time trends in control responses were examined by linear regression analysis (Wallenstein *et al.*, 1980). One-way analysis of variance (ANOVA) was used to detect treatment-related differences in the responses of each rat. Repeated measures analysis of variance (RMANOVA) was used to compare operant behavior between dose groups. Response ratios [vehicle/control (V/C), PCE/control (P/C) and PCE/vehicle (P/V)] for each of the eighteen 5-minute intervals in the operant sessions were calculated so that each rat served as its own control. These ratios were subjected to a mixed model RMANOVA with fixed factors of time and treatment, and a random factor of rat nested within treatment (Winer, 1971). In the event that time effects failed to satisfy multisample sphericity, test statistics for time effect and time x treatment interaction were

modified using the Huynh and Feldt adjustment factor (Huynh and Feldt, 1976). The RMANOVA was exclusive of rats 4B and 5B, both of whom failed to respond for the majority of the operant session following administration of 480 mg/kg PCE. Their exclusion did not change the conclusions drawn from variance analysis, but did modify p values. The minimum level of significance was set at $p \leq 0.05$ for all tests. On rare occasions, 5-minute intervals of operant data were lost due to equipment malfunction.

RESULTS

Operant Behavior

It was determined in a dose range-finding study that the administration of $\leq$ 80 mg/kg PCE by oral gavage had no effect on operant responding, whereas doses $\geq$ 640 mg/kg resulted in occasional gait disturbances and a complete failure to lever-press. Two intermediate doses, 160 and 480 mg/kg, were thus selected for study. The operant responses of six rats from each of the two dose groups are shown in Figures 1 and 2. The control response rates of the low and high dose groups ranged from 1.04-3.04 (mean $\pm$ SD, 1.88 $\pm$ 0.75) and 0.99-2.62 (1.63 $\pm$ 0.58) responses/second, respectively. Control response rates did not have a tendency to significantly increase or decrease over the course of the operant sessions.

Response rates of the two dose groups averaged 91.6 $\pm$ 5.2 (mean $\pm$ SD) and 111.0 $\pm$ 18.2% of control after administration of the 10 and 20% aqueous Alkamuls® vehicles, respectively. One-way analysis of variance detected differences between the control and post-vehicle responses of two of the twelve rats (3A and 6B). The failure of ANOVA to detect such a difference for rat 6A, when it is obvious that one exists, demonstrates its insensitivity to treatment-related effects when response rates are highly variable and effects are prevalent in only a portion of the overall operant session. Despite its insensitivity, variance analysis did detect PCE-induced changes from control

and post-vehicle responses that were much more prevalent in the high than in the low dose group.

Perchloroethylene-induced changes in responding of the low dose group were either not readily apparent (3A, 4A and 5A), restricted to the first 5-minute interval following dosing (1A) or attributable to gavage stress and the dosing vehicle (2A and 6A). In contrast, PCE's effects among the high dose group included the immediate suppression of responding for 15-30 minutes before a rapid recovery to control or post-vehicle response rates (1B, 2B, 3B and 6B) or the complete elimination of lever-pressing for the majority of the operant session (4B and 5B).

The repeated measures analysis of variance on V/C ratios failed to detect a significant time x treatment interaction ($p = 0.335$), but did detect significant treatment ($p = 0.016$) and time ($p = 0.025$) effects. In general, the administration of the 10% aqueous Alkamuls® vehicle reduced response rates, while the 20% vehicle increased response rates. This resulted in the high dose group having higher mean V/C ratios during sixteen of the eighteen 5-min operant session intervals. The vehicle effect was most apparent in the low dose group immediately after dosing, at which time the response rates of all six animals were lower than control rates. Because of the significant vehicle effect, RMANOVA on both P/C and P/V ratios was performed. The analysis of P/C ratios did not detect a significant time x treatment interaction ($p = 0.280$) or treatment effect ($p = 0.187$), but did detect a significant time effect ($p = 0.0005$). When P/V ratios were submitted to variance analysis, there was no significant time x treatment interaction ($p = 0.415$), but a highly significant treatment ($p = 0.001$) and time ($p = 0.023$) effect. The significant treatment effect stems from the suppression of operant responding by the 480 mg/kg dose of PCE, whereas the significant time effect is apparently due to the suppression of responding immediately after dosing, followed by increases in response ratios as the operant sessions progress. That RMANOVA detected a significant treatment effect on P/V and not P/C ratios is another indicator that vehicle

administration affected responding. The differential effect of treatment on the two types of ratios may be due to response rates after low dose administration differing more from control than from post-vehicle response rates. Response rates following low dose administration may have differed enough from control response rates that the effect of the high dose on P/C ratios was not significantly different.

Pharmacokinetics

The blood concentration versus time profiles following gavage with both PCE doses are presented in Figure 3. The long t½ of PCE relative to that of most volatile halocarbons was demonstrated by its presence in blood for several days after dosing. The pharmacokinetic parameter estimates for each dose group are summarized in Table 1. The differences in AUC and C_{max} between the two doses were nearly proportional to the difference in PCE dose. A 3-fold increase in dose resulted in AUC and C_{max} values that were 2.7 and 3.6-fold higher, respectively. Blood t½ did not differ with dose.

The blood and brain concentration versus time profiles for the 90 minutes immediately following dosing are presented in Figures 4 and 5, respectively. This 90-minute period corresponds to the time-course over which the SCOB of rats was monitored. Perchloroethylene was rapidly absorbed from the gastrointestinal (GI) tract as evidenced by its presence in blood and brain as early as 1 minute after dosing. Following a 10-15 minute phase of very rapid PCE uptake by the blood and brain, uptake rates slowed as peak levels were approached. At 15 minutes following administration of 160 mg/kg PCE, blood and brain levels had reached 94 and 80% of their maxima, respectively, after which they were relatively stable out to 90 minutes. In contrast, at 15 minutes following administration of the high dose, blood and brain concentrations were only 74 and 42%, respectively, of the concentrations at 90 minutes.

In both the blood and brain, the ratio of PCE concentrations between doses was greatest at 1 minute (9.5 in blood and 3.0 in brain). The blood PCE concentrations significantly differed between doses at all sampling times during the 90 minutes

immediately following dosing ($p \leq 0.002$). Despite a 3-fold difference in mean brain PCE concentrations at 1 minute, the difference was not statistically significant ($p = 0.072$) due to variability within the high dose group. Brain concentrations at 6, 15 and 20 minutes also were not significantly different between doses ($p = 0.108$, $p = 0.214$ and $p = 0.449$, respectively), but were so at all subsequent sampling times ($p \leq 0.007$).

Tissue dose time-courses for PCE were also determined in perirenal fat, liver and skeletal muscle (Figure 6). Prior to 30 minutes post dosing, concentrations in these tissues rarely differed between doses. Apparently, the rate of blood perfusion and lipid content of these tissues had a significant impact on PCE deposition. Based upon relative tissue concentrations at 1 and 6 minutes, the well perfused liver accumulated PCE at the highest rate, followed by the fat and muscle. By 15 minutes, fat concentrations had exceeded those in the liver. At 90 minutes post dosing, at which time behavioral monitoring was discontinued, PCE concentrations were greatest in the fat, followed by the liver, brain and muscle.

DISCUSSION

The investigation of possible relationships between solvent-induced central nervous system (CNS) effects and blood and brain solvent levels is important as a basis for predicting the time-course of solvent toxicity, understanding differential susceptibility and extrapolating risk across species and exposure scenarios (Orr *et al.*, 1976). With the exception of the large number of studies exploring the relationship between alcohol-induced CNS effects and blood ethanol levels (Sidell and Pless, 1971; Hurst and Bagley, 1972; Jones and Vega, 1972; Radlow and Hurst, 1985), few studies have attempted to relate solvent pharmacodynamics to pharmacokinetics. Bruckner and Peterson (1981) have demonstrated that blood and brain toluene concentrations are highly correlated with the degree of CNS depression in mice, as measured in tests of reflexes and unconditioned

performance. Also, Kishi *et al.* (1993) have reported a relationship between blood 1,1,2-trichloroethylene levels and shock avoidance performance decrements in rats. In studies of controlled human exposures, impaired body balance, eye tracking deficits and altered reaction times have been correlated with blood m-xylene or 1,1,1-trichloroethane concentrations (Riihimaki and Savolainen, 1980; MacKay *et al.*, 1987).

Except in studies of ethanol in which oral dosing has been used almost exclusively, the CNS effects of solvents have typically been investigated after, and to a much lesser extent during, inhalation exposure. In the present study, however, PCE was administered orally at doses that were 4.1 and 12.5% of the LD_{50} (Hayes *et al.*, 1986). This was necessitated by the immediate arrest of lever-pressing by exposure to even modest concentrations ($\approx$ 500 ppm) of PCE vapor. Based on previous studies recognizing the possible disruptive effects of odor and irritant properties of solvent vapors on SCOB (Balster *et al.*, 1982; Moser and Balster, 1986), we have attributed our observation of the cessation of lever-pressing to the odor or irritant properties of PCE. Burning of the eyes and irritation of the throat and nasal passages have been produced in humans by exposure to PCE in the 75-200 ppm range (ATSDR, 1990; NIOSH/OSHA, 1978), and the irritant potency of PCE in humans appears to be comparable to that of most industrial solvents. An exception appears to be the considerably less aversive 1,1,1-trichloroethane (Dick, 1988) and, while irritant potencies may differ for rats, it is noteworthy that 1,1,1-trichloroethane failed to disrupt operant behavior at concentrations well above those of PCE that abolished responding (Warren *et al.*, 1993). Similarly, the possibility that disruptive effects on operant behavior may be produced by local irritant effects of PCE on the GI tract cannot be dismissed, since patients receiving PCE as anthelmintic therapy have suffered abdominal cramps, nausea, vomiting and lesions of the oral and gastric mucosa (Reichert, 1983). However, the observation that lever-pressing was merely suppressed and not fully extinguished by PCE in most rats suggests

that GI irritation was not responsible for the behavioral effects observed in the present study.

The absorption and elimination pattern observed for PCE is characteristic of a lipid soluble, poorly metabolized chemical. Similar solvents have been shown to be rapidly and completely absorbed from the GI tract, with peak blood levels occurring from 2-15 minutes post dosing (Reitz *et al.*, 1982; D'Souza *et al.*, 1985; Putcha *et al.*, 1986). In the present study, PCE was quickly absorbed into the systemic circulation and taken up by body tissues. At 1 minute post dosing, PCE was present in not only the well perfused liver and brain, but also in the poorly perfused muscle and fat. This is in agreement with the observation of Dallas *et al.* (1994a) that peak levels for most tissues, including brain, occur at 1 minute following ia injection of PCE. Rapid uptake into tissues has also been reported following the autoradiographic analysis of mice after 10 min of ^{14}C-PCE inhalation (Ghantous *et al.*, 1986). Additionally, relatively high arterial blood concentrations of PCE were present only 2 minutes after the initiation of inhalation exposures to rats (Dallas *et al.*, 1994b). After oral administration, therefore, PCE follows a pattern of rapid uptake similar to that which follows administration by other routes.

Pegg *et al.* (1979) have reported the rapid and complete absorption of PCE after an oral bolus of 500 mg/kg in corn oil. These investigators observed a peak blood level of approximately 40 μg/ml, compared to a peak level in the current study of 78 μg/ml following the delivery of 480 mg/kg as an aqueous emulsion. This discrepancy in peak blood levels can be attributed to the slower rate of absorption from the corn oil vehicle, allowing time for tissue uptake and elimination processes to reduce blood levels. The t½ reported in the current study of approximately 9 hours is comparable to previously reported values of 7.43 and 8.27 hours for male SD rats (Pegg *et al.*, 1979; Dallas *et al.*, 1994a). Although an oral dose of 500 mg/kg PCE has reportedly resulted in the saturation of oxidative metabolism in the male SD rat (Pegg *et al.*, 1979), there was no

evidence of saturation in the current study as both the AUC and C_{max} were roughly proportional to dose.

Little information exists on PCE that allows for comparisons between rat and human data and oral and inhalation exposures. The ingestion of 8-10 ml of PCE (545-727 mg/kg) by a 6-year-old boy was followed by drowsiness, vertigo, agitation, hallucinations, somnolence and coma, from which he made a full recovery (Koppel *et al.*, 1985). One hour after ingestion, the PCE concentration in blood was 21.5 μg/ml, a level equal to the C_{max} observed following the oral administration of 160 mg/kg to rats. Also, the oral administration of PCE as an anthelmintic commonly resulted in narcotic effects, inebriation, perceptual distortion and exhilaration in patients receiving doses ranging from 60-86 mg/kg (assuming a body weight of 70 kg) (Wright *et al.*, 1937; Sandground, 1941; Haerer and Udelman, 1964). Regarding inhalation of PCE, Dallas *et al.* (1994a, 1994b) exposed rats to 500 ppm for 2 hours and measured blood and brain concentrations during and after exposure. At 90 minutes, the blood concentration was about 20 μg/ml. The maximum brain concentration was 173.9 μg/g, which is about midway between the maximum brain concentrations that followed administration of the two doses in our study.

One might expect that PCE exposure would not affect operant response rates until a threshold concentration in the brain is reached, after which rate changes may be biphasic or monotonic in a decreasing direction. Only response rate decreases would be expected in the current study since a FR schedule typically generates near maximal response rates. It was therefore anticipated that response rates would steadily decline as blood and brain PCE levels increased. Instead, it appears that there is no discernable relationships between blood or brain PCE levels and performance impairment. For example, there was little difference in brain levels between doses prior to the 30-minute sampling point, but drastic differences in operant behavior. Additionally, two-thirds of

the rats in the high dose group exhibited an immediate suppression of responding, followed by full recovery to control rates while blood and brain levels were still rising.

The rapid onset of response suppression suggests that the event which triggers behavioral effects occurs within the first 1 or 2 minutes after dosing. It is difficult to conclude, however, that the significant difference in behavior of the two dose groups was due to the relatively small difference in absolute brain concentrations at the 1-minute sampling point (0.72 $\pm$ 0.08 (mean $\pm$ SE) versus 2.14 $\pm$ 0.71 μg/g). Furthermore, it is evident that the behavior of the high dose group was not solely determined by brain levels at 1 minute, since concentrations up to 50-fold higher eventually occurred in the low dose group. Also, the blood level in the low dose group at 30 minutes (21.5 μg/ml) exceeded the blood level that occurred at 1 minute in the high dose group (19.1 μg/ml). It is possible that the response rate changes were related to the rate at which concentrations increased, rather than to the absolute blood or brain concentrations themselves. Such has been suggested for diazepam-induced impairment of psychomotor skills (Linnoila and Mattila, 1973) and for m-xylene-induced body sway (Riihimaki and Savolainen, 1980). It is conceivable that the slower rise in blood and brain concentrations seen after the lower dose of PCE allowed the CNS time to adapt, thereby minimizing PCE-induced changes in operant responding.

At the end of the operant sessions, brain concentrations in the high dose group were more than 100-fold higher than at 1 minute, and 8-fold higher than at 6 minutes. Blood levels were generally 2- to 3-fold higher when rats 1B, 2B, 3B and 6B were responding at control rates, than when their responding was severely suppressed. This phenomenon, which has been termed "acute adaptation," has been documented for ethanol and other CNS depressant drugs. Ellinwood *et al.* (1981a) have demonstrated that pentobarbital-induced impairment of subcritical tracking, pendulum eye tracking and standing steadiness in humans is much more marked during the initial phase of rapidly rising drug levels and is followed by rapid improvement of performance, despite rising

drug concentrations in blood. In yet another study by Ellinwood *et al.* (1981b), peak impairment of wheel tracking and digit-symbol substitution performance in humans was seen 20 minutes after oral diazepam when blood levels had reached less than two-thirds of their eventual plateau. There was a reduction in this impairment over the next 40 minutes even though blood diazepam levels continued to increase. In perhaps the most noted study of this phenomenon (LeBlanc *et al.*, 1975), the "acute adaptation" of rats to ethanol was demonstrated on a moving belt task. Impairment at a comparable brain level of ethanol was much greater 10 minutes after ip injection than at 30 minutes.

In a study similar to ours, Middaugh *et al.* (1992) examined lever responding in mice under a FR-20 food reinforcement schedule beginning 5 minutes after ip injection of ethanol. A dose of 1.5 g/kg reduced lever responding by 30% during the 0-4 min interval, but responding recovered to control rates by 5-8 minutes. Responding in mice given 2.0 g/kg was reduced 72 and 75% in the 5-8 and 9-12 minute intervals, respectively, but had recovered to control levels by 13-16 minutes. Based on the current study and the work of Middaugh *et al.* (1992), it appears that a very rapid adaptation may occur to effects on reinforced behaviors. It is known that functional tolerance or acute adaptation may develop more readily when the effect of the agent has a behavioral cost to the experimental animal, such as when it reduces the capability of a food-restricted animal to obtain a food reward (Jaffe, 1990).

It is evident from this study, as well as others, that the relationship between the pharmacokinetic distribution of solvents and their effects on the CNS is complex. Such complexity may stem from non-linearity between the administered dose of a solvent and the dose that reaches the brain; possible non-linearity between the arterial blood and brain concentrations; and little understood phenomena such as "acute adaptation." Given this complexity, it is becoming even more apparent that progress toward an understanding of mechanisms of solvent action in the CNS rests with performing studies that meet three criteria: 1) use of quantitative pharmacodynamic tests that allow for repeated measures

over time in order to ascertain a detailed time-course of effects, 2) use of highly sensitive and exact methods to assay blood and brain levels of solvents at repeated intervals, and ideally, 3) the measurement of blood and brain levels of solvents in those animals undergoing neurobehavioral testing (Orr *et al.*, 1976).

REFERENCES

(ATSDR) Agency for Toxic Substances and Disease Registry. (1990). *Case studies in environmental medicine: Tetrachloroethylene toxicity.* U.S.Department of Health and Human Services, Public Health Service, Centers for Disease Control, pp. 1-22.

(ATSDR) Agency for Toxic Substances and Disease Registry. (1992). *Toxicological Profile for Tetrachloroethylene.* TP-92/18. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Balster, R.L., Moser, V.C., and Woolverton, W.L. (1982). Concurrent measurements of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J. Pharmacol. Methods.* 8, 299-309.

Bruckner, J.V., and Peterson, R.G. (1981). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol. Appl. Pharmacol.* 61, 27-38.

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. (1993). Analyses of volatile C_2 haloethanes and haloethenes in tissues: sample preparation and extraction. *J. Chromatogr.* 612, 199-208.

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. (1994a). Development of a physiologically based pharmacokinetic model for perchloroethylene using tissue concentration-time data. *Toxicol. Appl. Pharmacol.* 128, 50-59.

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M., and Bruckner, J.V. (1994b). Use of a physiologically based model to predict systemic uptake and respiratory elimination of perchloroethylene. *Toxicol. Appl. Pharmacol.* 128, 60-68.

Dews, P.B. (1978). Epistemology of screening for behavioral toxicity. *Environ. Health Perspect.* 26, 37-42.

Dick, R.B. (1988). Short duration exposures to organic solvents: The relationship between neurobehavioral test results and other indicators. *Neurotoxicol. and Teratol.* 10, 39-50.

D'Souza, R.W., Bruckner, J.V., and Feldman, S. (1985). Oral and intravenous trichloroethylene pharmacokinetics in the rat. *J. Toxicol. Environ. Health.* 15, 587-601.

Ellinwood, E.H., Linnoila, M., Angle, H.V., Moore, J.W., Skinner, J.T., Easler, M., and Molter, D.W. (1981a). Use of simple tasks to test for impairment of complex skills by a sedative. *Psychopharmacology.* 73, 350-354.

Ellinwood, E.H., Linnoila, M., Easler, M.E., and Molter, D.W. (1981b). Onset of peak impairment after diazepam and after alcohol. *Clin. Pharmacol. Ther.* 30, no.4, 534-538.

Ferroni, C., Selis, L., Mutti, A., Folli, D., Bergamaschi, E., and Franchini, I. (1992). Neurobehavioral and neuroendocrine effects of occupational exposure to perchloroethylene. *Neurotoxicology.* 13, 243-247.

Fredriksson, A., Danielsson, B.R.G., and Eriksson, P. (1993). Altered behavior in adult mice orally exposed to tri- and tetrachloroethylene as neonates. *Toxicol. Lett.* 66, 13-19.

Ghantous, H., Danielsson, B.R.G., Dencker, L., Gorczak, J., and Vesterberg, O. (1986). Trichloroacetic acid accumulates in murine amniotic fluid after tri- and tetrachloroethylene inhalation. *Acta Pharmacol. Toxicol.* 58, 105-114.

Glowa, J.R., DeWeese, J., Natale, M.E., Holland, J.J., and Dews, P.B. (1983). Behavioral toxicology of volatile organic solvents. I. Methods: Acute effects. *J. Am. Coll. Toxicol.* 2, 175-185.

Gregersen, P. (1988). Neurotoxic effects of organic solvents in exposed workers: Two controlled follow-up studies after 5.5 and 10.6 years. *Am. J. Ind. Med.* 14, 681-701.

Haerer, A.F., and Udelman, H.D. (1964). Acute brain syndrome secondary to tetrachloroethylene ingestion. *Am. J. Psychiatry.* 12, 78-79.

Hake, C.L. and Stewart, R.D. (1977). Human exposure to tetrachloroethylene, inhalation and skin contact. *Environ. Health Perspect.* 21, 231-238.

Hayes, J.R., Condie, L.W., Borzelleca, J.F. (1986). The subchronic toxicity of tetrachloroethylene (perchloroethylene) administered in the drinking water of rats. *Fundam. Appl. Toxicol.* 7, 119-125.

Hurst, P.M., and Bagley, S.K. (1972). Acute adaptation to the effects of alcohol. *Quart. J. Stud. Alc.* 33, 358-378.

Huynh, H., and Feldt, L.S. (1976). Estimation of the Box correction for degrees of freedom from sample data in the randomized block and split-plot designs. *J. Educ. Stat.* 1, 69-82.

Jaffe, J.H. (1990). Drug addiction and drug abuse. In *Goodman and Gilman's, The Pharmacological Basis of Therapeutics* (Gilman, A.G. et al., eds.), 8th ed., pp. 522-531. Pergamon Press, New York.

Jones, B.M., and Vega, A. (1972). Cognitive performance measured on the ascending and descending limb of the blood alcohol curve. *Psychopharmacologia.* 23, 99-114.

Kishi, R., Harabuchi, I., Ikeda, T., Katakura, Y., and Miyake, H. (1993). Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans. *Br. J. Ind. Med.* 50, 470-480.

Kjellstrand, P., Holmquist, B., Jonsson, I., Romare, S., and Mansson, L. (1985). Effects of organic solvents on motor activity in mice. *Toxicology.* 35, 35-46.

Koppel, C., Arndt, I., Arendt, U., and Koeppe, P. (1985). Acute tetrachloroethylene poisoning - blood elimination kinetics during hyperventilation therapy. *J. Toxicol Clin Toxicol.* 23, 103-115.

Kulig, B.M. (1987). The effects of chronic trichloroethylene exposure on neurobehavioral functioning in the rat. *Neurotoxicol. Teratol.* 9, 171-178.

Lauwerys, R., Herbrand, J., Buchet, I.P., Bernard, A., and Graussin, J. (1983). Health Surveillance of workers exposed to tetrachloroethylene in dry-cleaning shops. *Int. Arch. Occup. Environ. Health.* 52, 69-77.

LeBlanc, A.E., Kalant, H., and Gibbins, R.J. (1975). Acute tolerance to ethanol in the rat. *Psychopharmacologia.* 41, 43-46.

Linnoila, M., and Mattila, M.J. (1973). Drug interaction on psychomotor skills related to driving: Diazepam and alcohol. *Eur. J. Pharmacol.* 5, 186-194.

Ludwig, H.R., Meister, M.V., Roberts, D.R., and Cox, C. (1983). Worker exposure to perchloroethylene in the commercial dry cleaning industry. *Am. Ind. Hyg. Assoc. J.* 44, 600-605.

Mackay, C.J., Campbell, L., Samuel, A.M., Alderman, K.J., Idzikowski, C., Wilson, H.K., and Gompertz, D. (1987). Behavioral changes during exposure to 1,1,1-trichloroethane: Time-Course and relationship to blood solvent levels. *Am. J. Ind. Med.* 11, 223-239.

Materna, B.L. (1985). Occupational exposure to tetrachloroethylene in the dry cleaning industry. *Am. Ind. Hyg. Assoc. J.* 46, 268-273.

Middaugh, L.D., Bao, K., and Shepherd, C.L. (1992). Comparative effects of ethanol on motor activity and operant behavior. *Pharmacol. Biochem. Behav.* 43, 625-629.

Moser, V.C., and Balster, R.L. (1986). The effects of inhaled toluene, halothane, 1,1,1-trichloroethane, and ethanol on fixed-interval responding in mice. *Neurobehav. Toxicol. Teratol.* 8, 525-531.

NCI. (1977). Bioassay of tetrachloroethylene for possible carcinogenicity. National Cancer Institute. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, DHEW Publ (NIH) 77-813.

NIOSH/OSHA. (1978). Occupational health guideline for tetrachloroethylene. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health. January 1992.

NOES. (1990). National Occupational Exposure Survey (1981-1983). U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Cincinnati, OH.

NTP. (1986). National Toxicology Program - technical report series no.311. Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS No. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH publication no. 86-2567.

Orr, J., Dussault, P., Chappel, C., Goldberg, L., and Reggiani, G. (1976). Relation between drug-induced central nervous system effects and plasma levels of diazepam in man. *Mod. Probl. Pharmacopsych.* 11, 57-67.

Pegg, D.G., Zempel, J.A., Braun, W.H., and Watanabe, P.G. (1979). Disposition of tetrachloro(^{14}C)ethylene following oral and inhalation exposure in rats. *Toxicol. Appl. Pharmacol.* 51, 465-474.

Putcha, L., Bruckner, J.V., D'Souza, R.D., Desai, F., and Feldman, S. (1986). Toxicokinetics and bioavailability of oral and intravenous 1,1-dichloroethylene. *Fund. Appl. Toxicol.* 6, 240-250.

Radlow, R., and Hurst, P.M. (1985). Temporal relations between blood alcohol concentration and alcohol effect: an experiment with human subjects. *Psychopharmacology*. 85, 260-266.

Reichert, D. (1983). Biological actions and interactions of tetrachloroethylene. *Mutat. Res.* 123, 411-429.

Reitz, R.H., Fox, T.R., Ramsey, J.C., Quast, J.F., Langvardt, P.W., and Watanabe, P.G. (1982). Pharmacokinetics and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. *Toxicol. Appl. Pharmacol.* 62, 190-204.

Riihimaki, V., and Savolainen, K. (1980). Human exposure to m-xylene. Kinetics and acute effects on the central nervous system. *Ann. Occup. Hyg.* 23, 411-422.

Rowe, V.K., McCollister, D.D., Spencer, H.C., Adams, E.M., and Irish, D.D. (1952). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects. *AMA Arch. Ind. Hyg. Occup. Med.* 5, 566-579.

Sandground, J.H. (1941). Coma following medication with tetrachloroethylene. *J. Am. Med. Assoc.* 117, 440-441.

Savolainen, H., Pfaffli, P., Tengen, M., and Vainio, H. (1977). Biochemical and behavioral effects of inhalation exposure to tetrachloroethylene and dichloromethane. *J. Neuropath. Exp. Neurology.* 36, 941-949.

Seeber, A. (1989). Neurobehavioral toxicity of long-term exposure to tetrachloroethylene. *Neurotoxicol. Teratol.* 11, 579-583.

Sidell, F.R., and Pless, J.E. (1971). Ethyl alcohol: Blood levels and performance decrements after oral administration to man. *Psychopharmacologia.* 19, 246-261.

Stewart, R.D., Baretta, E.D., Dodd, H.C., and Torkelson, T.R. (1970). Experimental human exposure to tetrachloroethylene. *Arch. Environ. Health.* 20, 225-229.

Wallenstein, S., Zucker, C.L., and Fleiss, J. (1980). Some statistical methods useful in circulation research. *Circ. Res.* 47, no.1, 1-9.

Warren, D.A., Dallas, C.E., Reigle, T.G., and Christmus, W.H. (1993). Behavioral changes during 1,1,1-trichloroethane (TRI) inhalation in rats: Relationship to brain and blood levels. *The Toxicologist.* 13, 248.

Winer, B.J. (1971). *Statistical Principles in Experimental Design.* 2nd ed., McGraw, New York.

Wood, R.W., Rees, D.C., and Laties, V.G. (1983). Behavioral effects of toluene are modulated by stimulus control. *Toxicol. Appl. Pharmacol.* 68, 462-472.

Wright, W.H., Bozicevich, J., and Gordon, L.S. (1937). Studies on oxyuriasis. V. Therapy with single doses of tetrachloroethylene. *J. Am. Med. Assoc.* 109, no.8, 570-573.

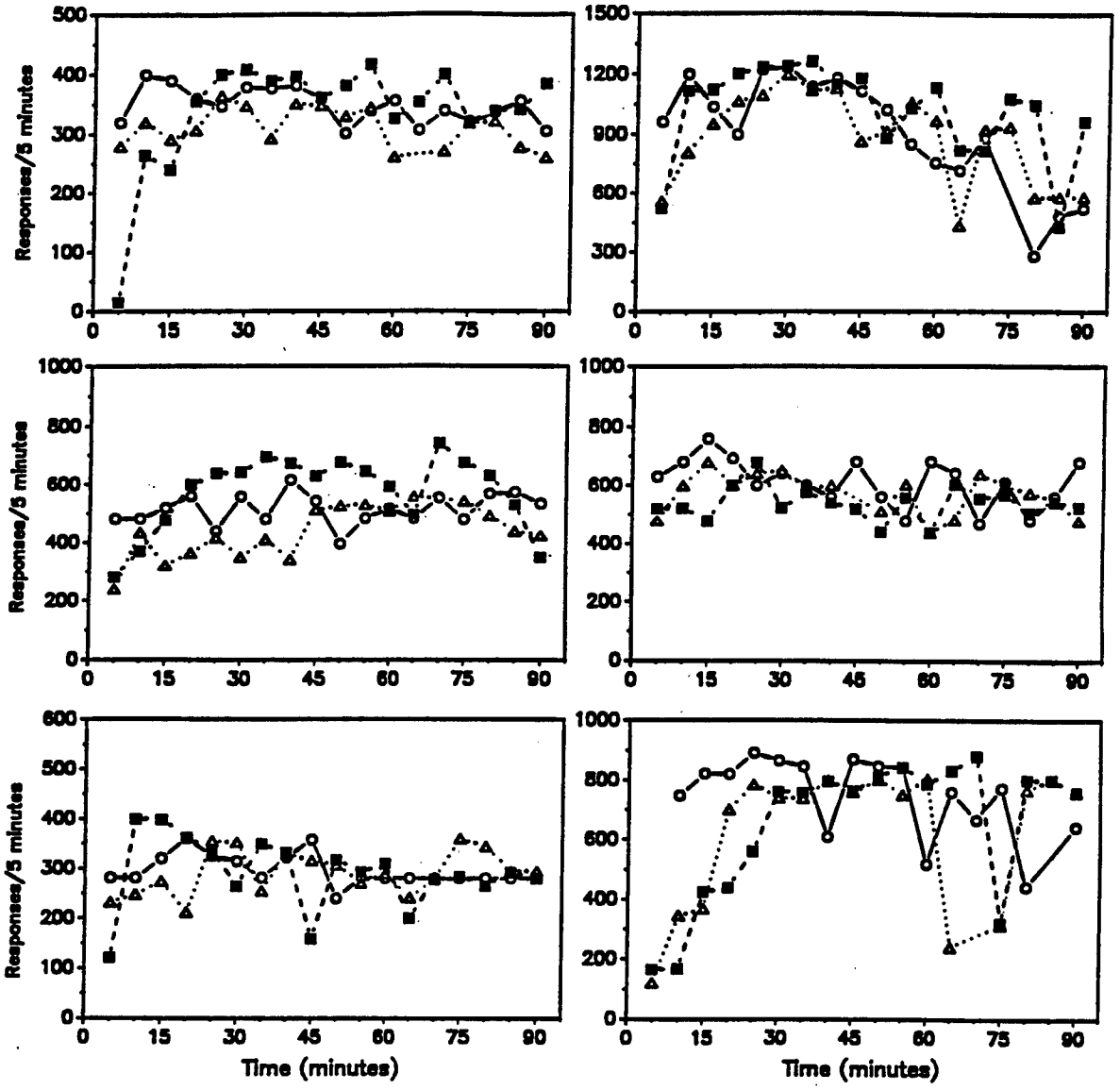

Figure 1. The operant responses of six rats following three treatments: no treatment or control (○); the oral administration of a 10% Alkamuls® vehicle (△); the oral administration of 160 mg/kg PCE (■).

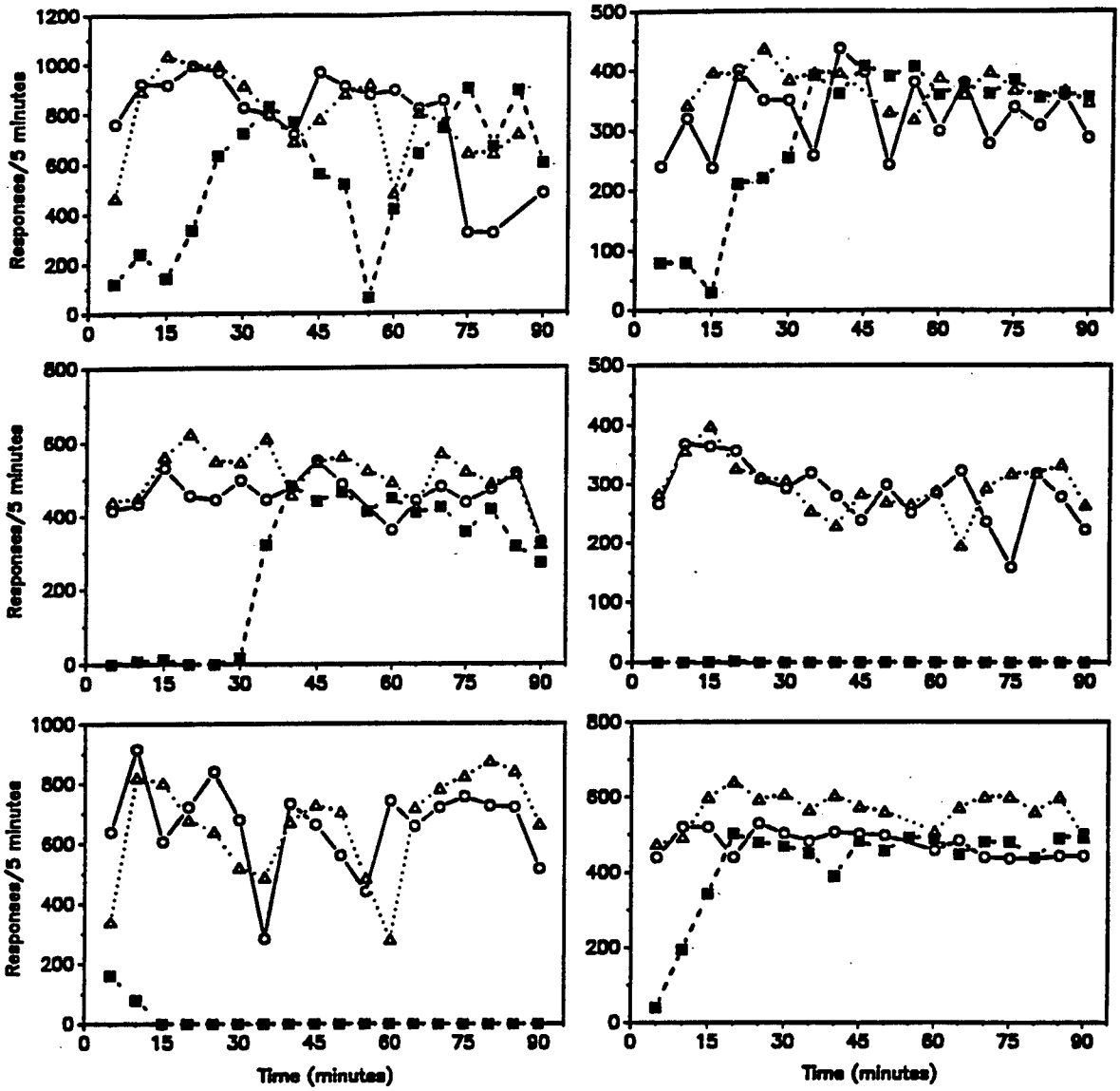

Figure 2. The operant responses of six rats following three treatments: no treatment or control (○); the oral administration of a 20% Alkamuls® vehicle (△); the oral administration of 480 mg/kg PCE (■).

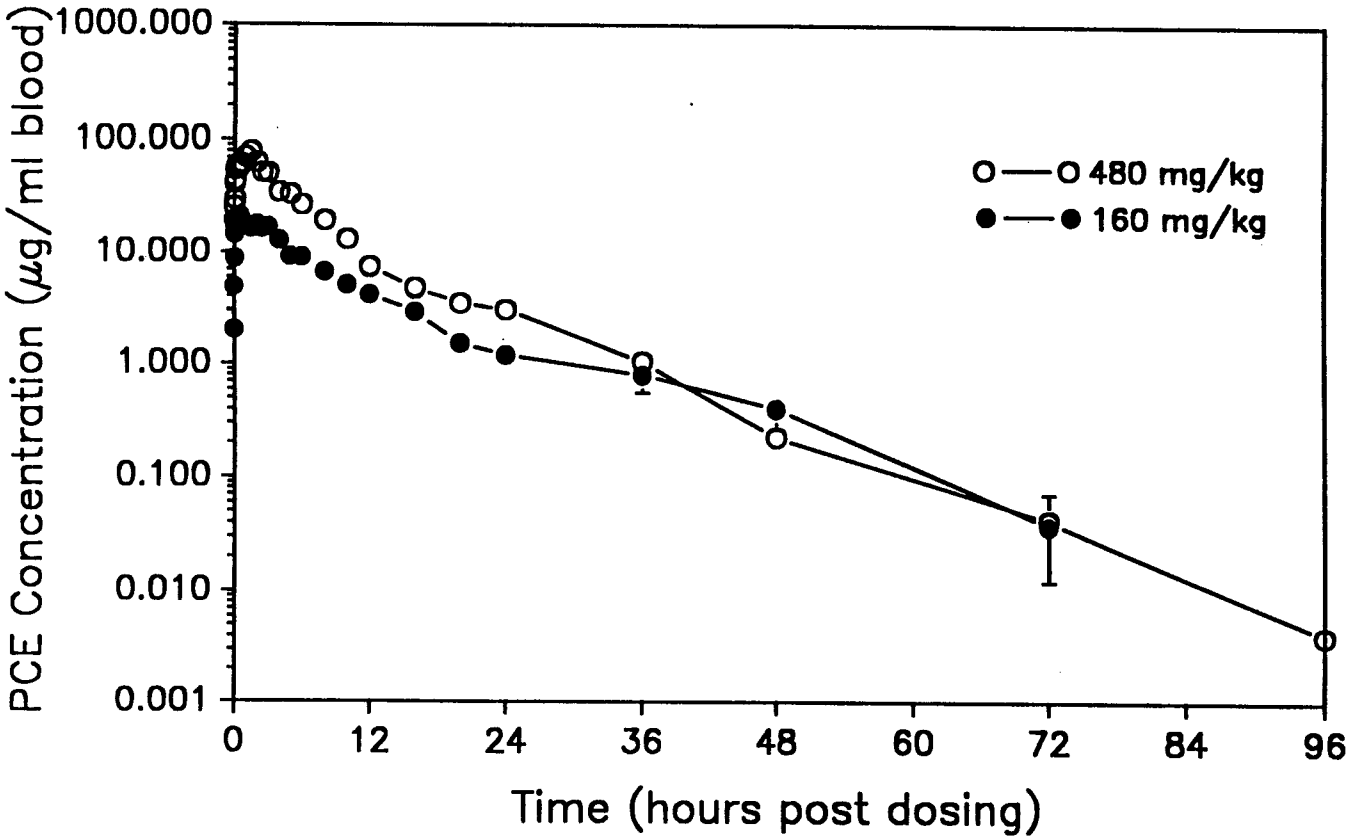

Figure 3. Blood PCE concentration versus time profiles following the oral administration of 160 and 480 mg/kg PCE. Each profile represents the mean ± SE of six rats per dose. The concentration of PCE in blood at 96 hours following administration of 160 mg/kg was below the limit of detection. SE bars are obscured by symbols in most cases.

Table 1. Pharmacokinetic Parameters Following Oral Administration of Perchloroethylene to Rats[a]

Dose (mg/kg)	AUC ($\mu g \cdot min/ml$)[b]	$t_{1/2}$ (min)[c]	C_{max} ($\mu g/ml$)[d]
160	9508 ± 730	504 ± 46	21.5 ± 2.8
480	25256 ± 1162	555 ± 78	78.0 ± 6.2

[a]Values are the Mean ± SE of 6 rats per dose.
[b]Area under the curve that describes the concentration of PCE in blood as a function of time.
[c]Elimination half-life or the amount of time required for the blood concentration of PCE to be reduced by 50%.
[d]Maximum concentration of PCE in carotid arterial blood.

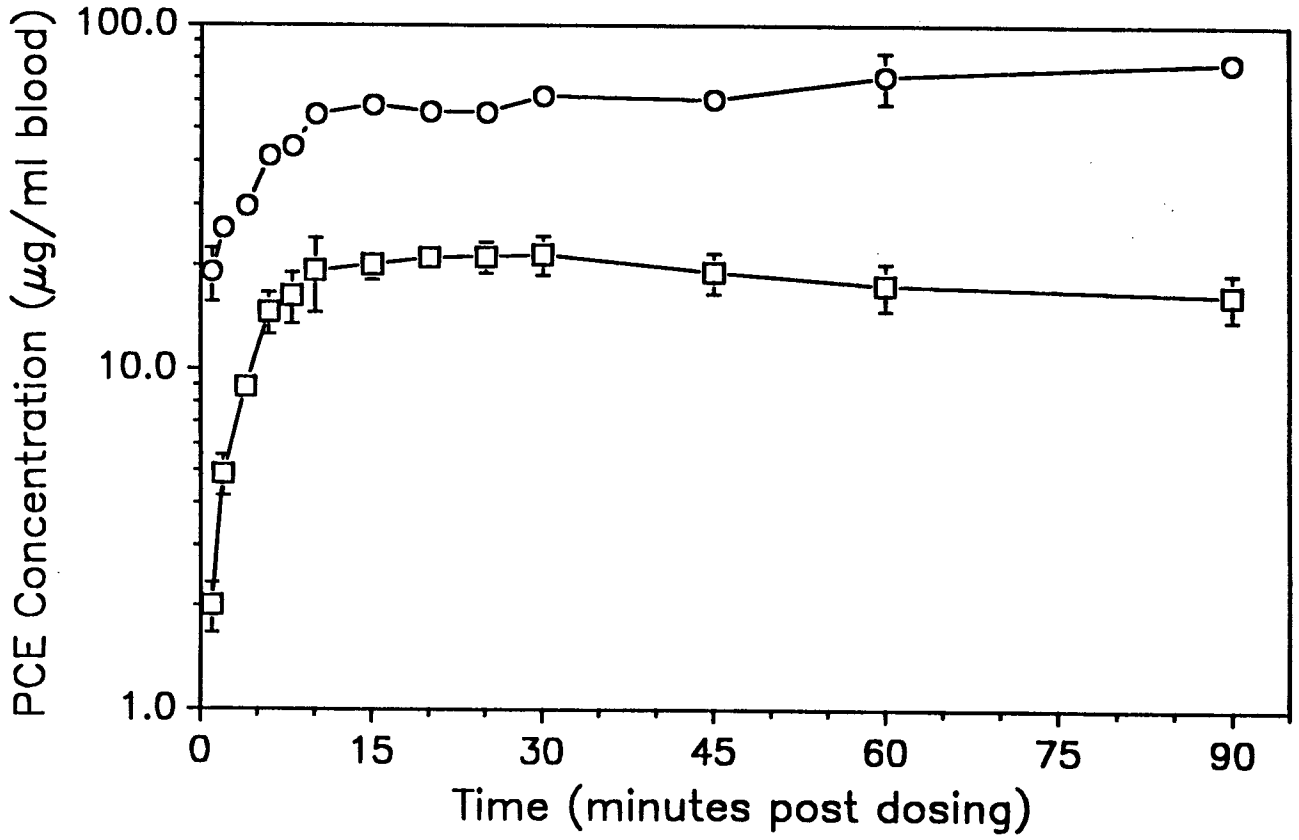

Figure 4. The uptake of PCE in blood during the 90 minutes immediately following oral administration of 160 (□) and 480 mg/kg PCE (O). Each profile represents the mean ± SE of six rats per dose. SE bars are obscured by symbols in most cases.

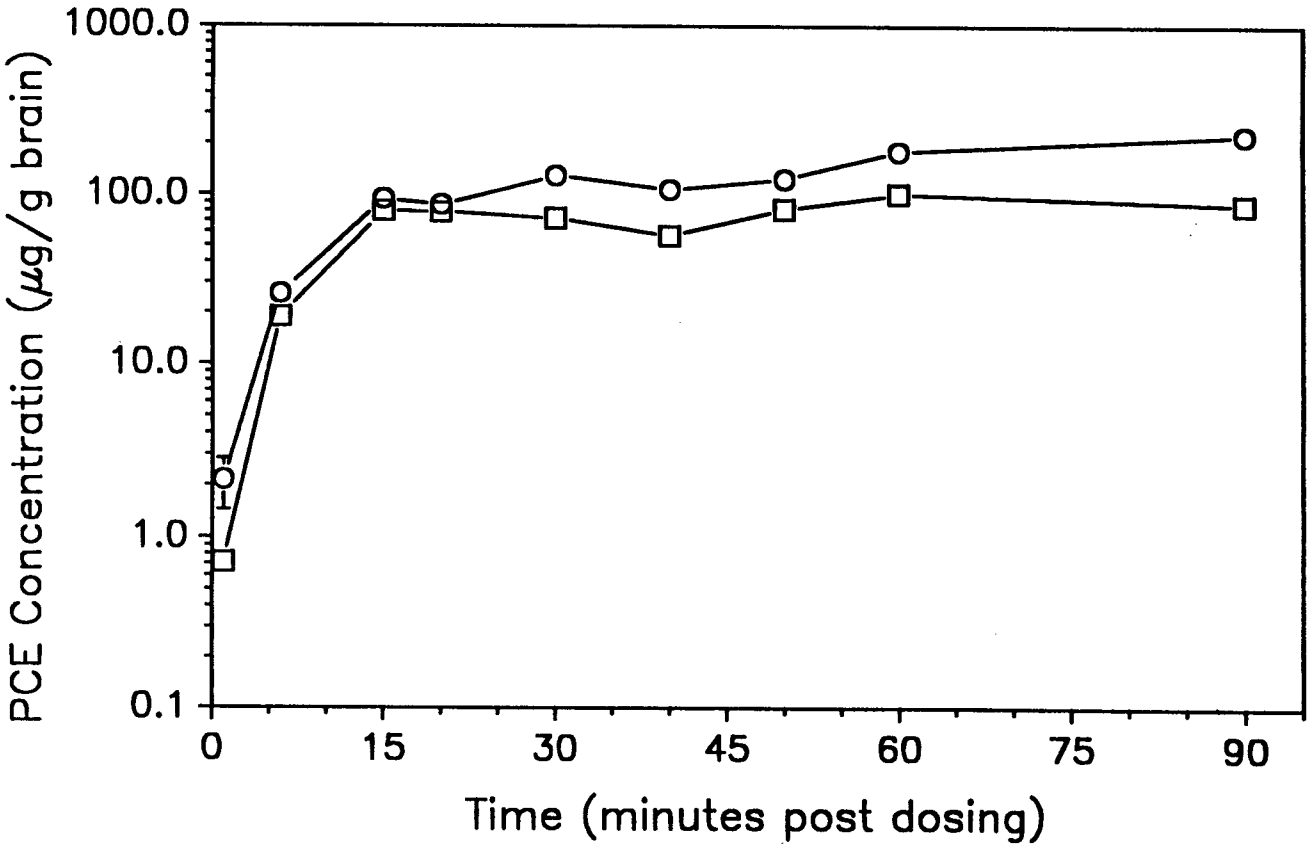

Figure 5. The uptake of PCE in brain during the 90 minutes immediately following oral administration of 160 (□) and 480 mg/kg PCE (○). Each data point represents the mean ± SE of six rats. SE bars are obscured by symbols in most cases.

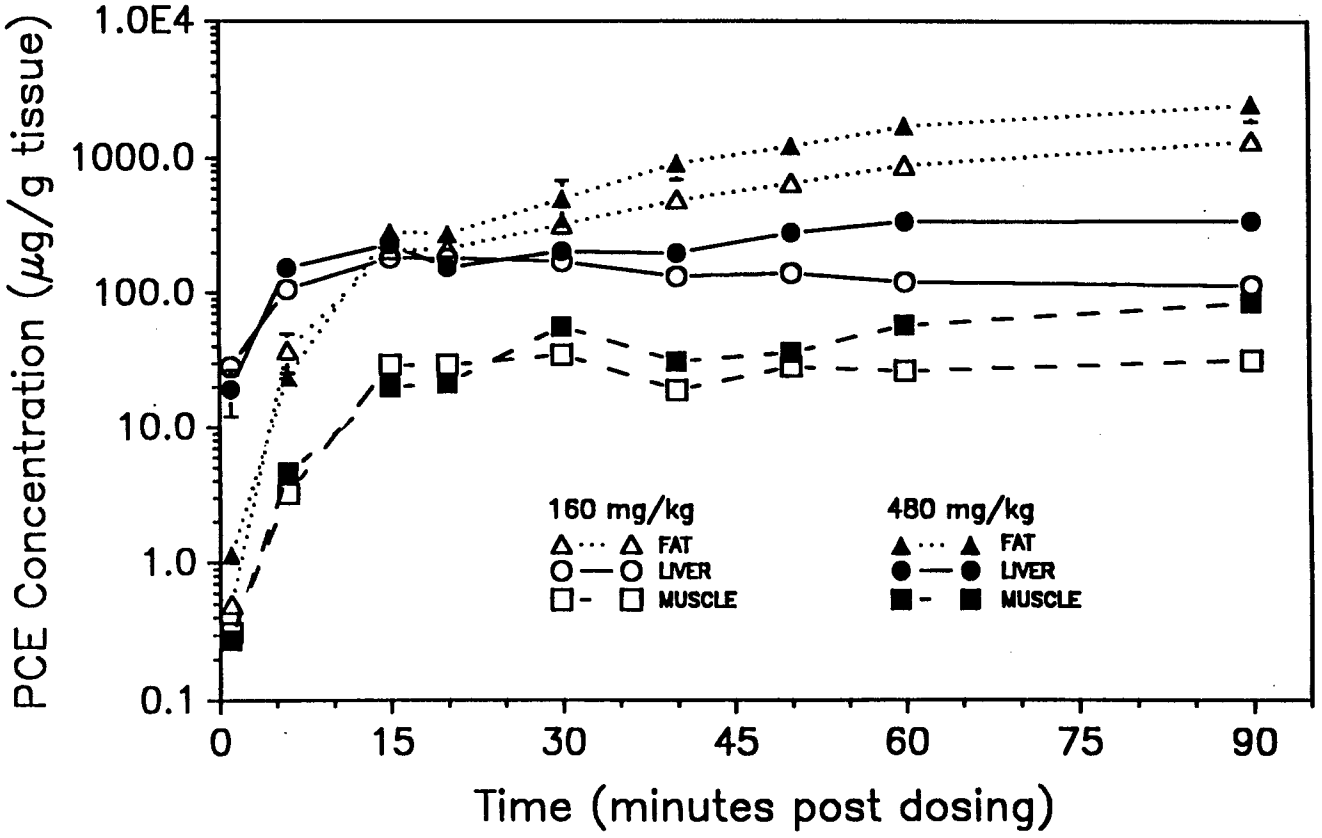

Figure 6. The uptake of PCE in perirenal fat, liver and skeletal muscle during the 90 minutes immediately following oral administration of 160 and 480 mg/kg PCE. Each data point represents the mean ± SE of six rats. SE bars are obscured by symbols in most cases.

SCHEDULE-CONTROLLED OPERANT BEHAVIOR OF RATS DURING 1,1,1-TRICHLOROETHANE INHALATION: RELATIONSHIP TO BLOOD AND BRAIN SOLVENT CONCENTRATIONS[1]

[1]Warren, D.A., Reigle, T.G., Christmus, W.H., Muralidhara, S., and Dallas, C.E. To be submitted to *Neurotoxicology and Teratology*.

ABSTRACT

The central nervous system is the principal target of 1,1,1-trichloroethane (TRI), and several studies of this volatile solvent have demonstrated effects on learned animal behaviors. There have been no attempts, however, to quantitatively relate such effects to blood or target organ (i.e., brain) solvent concentrations. Therefore, Sprague-Dawley rats trained to lever-press for evaporated milk on a variable interval-30 second reinforcement schedule were placed in an operant test cage and exposed to clean air for 20 minutes, followed by a single concentration of TRI vapor (500-5000 ppm) for 100 minutes. Additional rats were exposed to equivalent TRI concentrations for 10, 20, 40, 60, 80 or 100 minutes to determine blood and brain concentration versus time profiles. Inhalation of 1000 ppm slightly increased operant response rates, whereas 2000, 3500 and 5000 ppm decreased operant response rates in a concentration- and time-dependent manner. Accumulation of TRI in blood and brain was rapid and concentration-dependent, with the brain concentration roughly twice that of blood. Plots of blood and brain TRI concentrations against operant performance showed responding in excess of control rates at low concentrations, and decreasing response rates as concentrations increased. Linear regression analyses indicated that blood and brain concentrations were strongly correlated with, and equally predictive of, operant performance. Neurobehavioral toxicity in laboratory animals, as measured by changes in operant performance, can therefore be quantitatively related to internal measures of TRI exposure to enhance its predictive value for human risk assessment.

INTRODUCTION

1,1,1-Trichloroethane (methyl chloroform; TRI) is a volatile organic solvent used in large quantities as a dissolvent, metal degreaser, chemical intermediate and component

of consumer products. Originally produced as a safer alternative to other chlorinated solvents, the acute and chronic toxicities of TRI are relatively low. There is, however, a risk of toxic effects to those that encounter TRI in high concentrations in the workplace or recreationally abuse the solvent. It is thus noteworthy that an estimated 2.5 million U.S. workers are potentially exposed occupationally (ATSDR, 1994), and that solvent abuse has become a significant public health problem (Evans and Balster, 1991).

Severe exposures of humans to TRI have resulted in sensitization of the heart to epinephrine-induced arrhythmias and mild hepatorenal effects (ATSDR, 1994), but the central nervous system (CNS) is considered the principal target. Acute exposures to volunteers have produced impaired performance in tests of manual dexterity, eye-hand coordination, perceptual speed and reaction time (Gamberale and Hultengren, 1973; Mackay *et al.*, 1987). Some of these deficits have occurred at concentrations at or below the 8-hour, time-weighted average workplace exposure limit of 350 ppm. Less subtle effects such as lightheadedness and imbalance are usually observed at concentrations $\geq$ 900 ppm (Torkelson *et al.*, 1958; Stewart *et al.*, 1961).

Studies of operant behavior are thought to reflect effects of TRI in laboratory animals comparable to psychomotor changes in humans. Accordingly, TRI has produced rate changes in the food-reinforced lever-pressing of mice (Balster *et al.*, 1982; Moser and Balster, 1986), altered performance on a match-to-sample discrimination task in baboons (Geller *et al.*, 1982) and impaired the ability of rats to avoid shock by lever-pressing (Mullin and Krivanek, 1982). These studies demonstrate that TRI's effects are concentration- and time-dependent, thereby inferring dependence on blood and target tissue (i.e., brain) doses. In no case, however, was blood or brain concentration measured for correlation with observed effects.

Except for the large number of studies exploring the relationship between alcohol-induced CNS effects and blood ethanol levels (Sidell and Pless, 1971; Hurst and Bagley, 1972; Jones and Vega, 1972; Radlow and Hurst, 1985), few studies have attempted to

correlate solvent pharmacodynamics with pharmacokinetics. This is unfortunate since such studies provide a basis for predicting the time-course of solvent toxicity, understanding differential susceptibility and extrapolating risk across species and exposure scenarios (Orr *et al.*, 1976). We report results of an investigation designed to examine the relationship between blood and brain TRI concentrations and changes in the schedule-controlled operant behavior (SCOB) of rats. 1,1,1-Trichloroethane was selected for study because it undergoes minimal metabolism and is psychoactive in humans and rodents. Furthermore, blood TRI concentration data are available from both controlled human and animal exposures. Schedule-controlled operant behavior was employed since it allows for repeated, uninterrupted measures over time that are sufficiently quantitative for correlation with measures of internal dose.

MATERIALS AND METHODS

Chemicals: 1,1,1-Trichloroethane of 97% + purity was obtained form Aldrich Chemical Co., Inc. (Milwaukee, WI). Burdick and Jackson Brand, High Purity Solvent isooctane was obtained from Baxter Healthcare Corp. (Muskegon, MI).

Animals: Male Sprague-Dawley (SD) rats (275-350 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were housed two per cage in suspended wire-bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hour light-dark cycle (light: 0700-1900 hr). Rats were acclimated for at least 7 days prior to use, during which time food (Purina Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*. All experiments were conducted during the light phase of the light-dark cycle.

TRI Vapor Generation: 1,1,1-Trichloroethane exposures were conducted in a 1.0 M^3 Rochester-type dynamic flow inhalation chamber operating at a flow rate of 14 ft^3/minute, under a negative pressure of 20″ H_20. Nitrogen was passed through a glass

dispersion flask of liquid TRI from which solvent vapor entered the chamber's influent air stream. A heating mantle was placed around the dispersion flask to generate vapor concentrations $\geq$ 2000 ppm. The flask was enclosed in a plexiglass safety box under constant negative pressure. Exhaust air from the inhalation chamber and safety box was vented through HEPA and activated charcoal filters prior to its release into the environment. Vapor concentrations were continuously monitored with a Miran 1B2 portable infrared spectrophotometer ($\lambda = 9.4\ \mu m$) (The Foxboro Co., East Bridgewater, MA) interfaced with a microcomputer-based Foxboro DL 332F Datalogger (Metrosonics Inc., Rochester, NY). The Miran was calibrated with a closed loop system (The Foxboro Co., East Bridgewater, MA) and the calibration accuracy verified just prior to each exposure with liquid TRI injections that volatilized to produce concentrations spanning the calibration range. Target vapor concentrations were reached within 2-5 minutes and thereafter exhibited $\pm$ 5% random fluctuation. Occasional adjustments in nitrogen flow and heating mantle temperature were necessary to maintain target concentrations.

Behavioral Apparatus: Operant sessions took place in a slotted test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) positioned inside a 1.0 M^3 Rochester-type dynamic flow inhalation chamber that served not only to expose animals, but also to isolate them from extraneous stimuli. This test cage was equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant performance schedule and recorded the number of responses and reinforcers in each 5-minute interval of the operant session.

Operant Behavior: Rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cobb bedding and stainless steel wire lids. Rats were food-restricted

(10 ± 0.25 g/day) during a period in which they were trained to lever-press for undiluted evaporated milk (0.08 ml for 7 seconds/reinforcement) on a variable interval-30 second (VI-30) reinforcement schedule. Initially, rats were manually reinforced in 30-minute sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the mean interval between reinforcer availability was gradually increased to 30 seconds and the session length extended to 120 minutes. Rats responded in daily 120-minute sessions spaced 24 hours apart until their response rates stabilized, a process requiring 15-20 days. The criterion for stable behavior was four successive sessions in which the number of responses/second varied by less than 15% from the 4-day mean rate. Once rats met the stability criterion, their behavior was monitored during exposure to clean air for 20 minutes, followed by a single concentration of TRI vapor (500, 1000, 2000, 3500 or 5000 ppm) for 100 minutes. Five chemically-naive rats were exposed to each TRI concentration.

Blood and Brain Sampling: Rats were transferred to individual wire-bottom cages (36 x 20 x 20 cm), food restricted (10 ± 0.25 g/day), and orally gavaged with 10 ml/day (two 5 ml boli given 1 hour apart) of undiluted evaporated milk for 15-20 days. This provided a diet comparable to that of rats in the behavioral study. At the end of the 15-20 day period, groups of five to twenty rats were placed in partitioned wire-mesh cages positioned in the same inhalation chamber employed in the behavioral study. Thirty rats were exposed to 1000 ppm TRI, five for each of the following durations: 10, 20, 40, 60, 80 and 100 minutes. Thirty rats were similarly exposed to 2000, 3500 and 5000 ppm TRI. At appropriate times during exposure, rats were extracted from the chamber through portals in the glass door with only a slight, transient decline in chamber concentration. The extracted rats were sacrificed by cervical dislocation after which blood samples (0.1-1.0 ml, depending upon the anticipated blood concentration) were quickly obtained by closed chest cardiac puncture with a 21 gauge needle and a 10 ml syringe. The brain (≈ 1.0 g samples) was removed within 2.5-3.0 minutes from each

rat and like the blood sample, immediately placed into a chilled scintillation vial containing 1.0 ml of 0.9% saline and 4.0 ml of isooctane.

TRI Analysis: Blood and brain samples were homogenized as quickly as possible (5-10 seconds) with an Ultra-Turrax® homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of TRI. Samples were then vortex-mixed for 30 seconds. The homogenates were centrifuged at 2500 x g for 10 minutes at 4°C in the capped scintillation vials. An aliquot of the isooctane layer (3-20 μl) was either transferred directly to a 20 ml headspace vial or first diluted with isooctane. The vials were capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly. A Perkin-Elmer Model 8500 gas chromatograph with a HS-101 headspace autosampler and electron capture detector was used for the analysis of TRI in blood and brain. Analyses were carried out using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 70°C; injection port temperature, 150°C; column temperature, 80°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/minute. 1,1,1-Trichloroethane concentrations were calculated from daily prepared standard curves made by diluting various amounts of TRI in isooctane and corrected for the percent recovery characteristic of blood and brain samples. The percent recovery and blood/brain extraction procedures have been described by Chen *et al.* (1993). The limit of detection for TRI was approximately 1 ng in 20 ml air.

Data Analysis: Areas-under-blood- and brain-concentration versus time curves (AUC) were calculated by the trapezoidal rule (Rowland and Tozer, 1980). Maximum blood and brain concentrations (C_{max}) were obtained by visual inspection of pharmacokinetic data. Each animals control behavior was calculated as the average number of lever-presses in each 5-minute interval of the four operant sessions used to meet the stability criterion. Time trends in control behavior were examined by linear regression analysis

(Wallenstein *et al.*, 1980). The effect of TRI in individual rats was determined by calculating an operant response ratio (lever-presses during a test session/lever-presses during control behavior; TRI/Control) for each 5-minute interval of a test session. Operant response data are presented as the mean ± standard error (SE) of these individual determinations and as a function of exposure concentration. Operant response ratios during TRI exposure were subjected to a mixed-model repeated measures analysis of variance (RMANOVA) with fixed factors of time and concentration, and a random factor of rat nested within concentration (Winer, 1971). In the event that time effects failed to satisfy multisample sphericity, test statistics for time effect and time x concentration interaction were modified using the Huynh and Feldt adjustment factor (Huynh and Feldt, 1976). One-way analysis of variance (ANOVA) and a *post hoc* "least significant difference" (LSD) test were applied to the mean operant response ratios. Paired t-tests were also used to compare the number of responses under control and test conditions. Pooling data from four exposure groups (1000, 2000, 3500 and 5000 ppm), blood and brain concentrations were plotted against each other, as well as against mean operant response ratios measured during the 5-minute intervals immediately preceding and following blood and brain collection. The resulting scatter plots, as well as the curve relating exposure concentration to operant responding, were subjected to least squares linear regression analysis and the degree of correlation measured by comparing correlation coefficients to values in a t-distribution table (Gad and Weil, 1986). EC_{50} values (blood, brain and exposure concentrations expected to decrease responding by 50%) were determined by solving equations for the regression lines, and the confidence interval (CI) was defined using SAS (SAS Institute, Cary, NC). The minimum level of significance was set at $p \leq 0.05$ for all tests. Measures of variation are standard deviations (SD) unless otherwise specified.

RESULTS

Operant Behavior

Control response rates of the 500, 1000, 2000, 3500 and 5000 ppm exposure groups were 0.31 ± 0.15, 0.31 ± 0.17, 0.46 ± 0.10, 0.36 ± 0.08 and 0.44 ± 0.11 responses/second, respectively. Control response rates did not have a tendency to significantly increase or decrease over the course of operant sessions. Response rates during the 20 minutes preceding TRI exposure were ± 25% of control rates for 20/25 rats, and within ± 2 SD of control rates for the remaining five. Nonetheless, for the 500 ($t = 2.346$, $df = 19$, $p = 0.030$) and 5000 ppm ($t = 3.054$, $df = 19$, $p = 0.007$) exposure groups, paired t-tests detected that response rates during the 20 minutes preceding TRI exposure were higher than control response rates for the same period.

Mean operant response ratios (± SE) of the five exposure groups over time are shown in Figures 1a-1e. Operant response ratios during TRI exposure averaged 1.20 ± 0.15, 1.20 ± 0.17, 0.83 ± 0.22, 0.53 ± 0.14 and 0.15 ± 0.16 for the 500, 1000, 2000, 3500 and 5000 ppm exposure groups, respectively, on which basis the dose-response curve in Figure 2 was composed (EC_{50} = 3577 ppm, 95% CI = 2864-4253 ppm). Repeated measures analysis of variance detected a significant time x concentration interaction ($F(76, 380) = 1.50$, $p = 0.037$) and concentration effect ($F(4, 20) = 28.63$, $p < .0001$), but no time effect ($F(19,380) = 1.44$, $p = 0.162$). One-way ANOVA followed by a *post hoc* LSD test also detected the effect of concentration ($F(4, 95) = 140.20$, $p < 0.0001$), as well as that operant response ratios of all exposure groups during TRI inhalation differed from unity ($p < 0.01$) and each other ($p < 0.01$), with the exception of the 500 and 1000 ppm exposure groups which did not differ. In addition, paired t-tests detected response rate increases during exposure to 500 ($t = 5.103$, $df = 99$, $p < 0.0001$) and 1000 ppm ($t = 4.376$, $df = 99$, $p < 0.0001$), and response rate decreases during exposure to 2000 ($t = 4.193$, $df = 99$, $p < 0.0001$),

3500 (t = 12.245, df = 99, p < 0.0001) and 5000 ppm (t = 25.059, df = 99, p < 0.0001). Despite statistical indices that 500 ppm increased operant responding, derivation of this conclusion was hindered by the elevated response rate exhibited by the 500 ppm group during the 20-minute pre-exposure period (Figure 1a). Mean operant response ratios of the 500 and 1000 ppm exposure groups were nearly identical, but subjects in the latter group demonstrated more variable response rates during exposure, which may itself be indicative of increased toxicity. Response rate decreases were concentration- and time-dependent. Decreased response rates were not exhibited until after 60 minutes of exposure to 2000 ppm. However, inhalation of 3500 ppm resulted in an immediate, but gradual decline in the average response rate to approximately 50% of control, while inhalation of 5000 ppm produced a precipitous drop in the response rate to approximately 15% of control. Only during exposure to this highest concentration did rats cease responding for periods in excess of 5 minutes, during which the animals typically assumed an immobile posture in close proximity to the lever. Rats did not exhibit ataxia or signs of irritation of the eyes, nose or mouth, and animals exposed to 5000 ppm returned to pre-exposure response rates within 15-25 minutes of exposure cessation.

Pharmacokinetics

Changes in TRI concentrations in the blood and brain as a function of degree and duration of exposure are shown in Figures 3a and 3b, respectively. These concentration versus time profiles correspond to the time-course over which the SCOB of rats was monitored. 1,1,1-Trichloroethane was rapidly absorbed from the lung as evidenced by its substantial presence in blood and brain as early as 10 minutes after dosing. Following a 20 to 40-minute phase of very rapid TRI uptake by the blood and brain, the rate of increase slowed as near steady-state equilibria between alveolar, blood and brain TRI concentrations were approached. Steady-state equilibria were apparently achieved during the 1000 and 2000 ppm exposures, while TRI concentrations were still increasing after

100 minutes of exposure to 3500 and 5000 ppm. Maximum blood and brain concentrations were proportional to exposure concentrations, while AUCs became slightly less than proportional at 3500 and 5000 ppm (Figures 4 and 5).

As expected for a well-perfused and lipid-rich organ such as the brain, its pattern of TRI accumulation was very similar to that of the blood. The scatter plot relating mean blood and brain solvent concentrations (Figure 6) clearly illustrates that brain levels of TRI increase in proportion to blood levels ($\approx$ 2:1), making blood and brain solvent concentrations highly correlated ($r = 0.983$, $df = 21$, $t = 24.53$, $p < 0.001$). These findings strongly suggest that blood and brain TRI concentrations are equally suited to relate to operant performance measures.

Pharmacodynamic/Pharmacokinetic Correlation

The scatter plots relating mean blood and brain solvent concentrations to operant performance are presented in Figure 7. Responding was in excess of control rates at low blood and brain concentrations, and decreased as blood and brain concentrations increased. Linear regression analyses indicated that blood ($r = -0.75$, $t = 0.4375$, $df = 19$, $p < 0.001$) and brain ($r = -0.75$, $t = 0.4345$, $df = 19$, $p < 0.001$) concentrations were strongly correlated with, and equally predictive of, operant performance. Blood and brain EC_{50} values were 41.6 μg/ml and 86.6 μg/g, respectively.

DISCUSSION

Comparisons between the present and previous studies of TRI's effects on operant behavior are limited by the use of different species, exposure parameters and reinforcement schedules. However, the inhalation EC_{50} of 3577 ppm obtained in the present study is comparable to those reported in two previous investigations of fixed ratio responding in mice during TRI exposure (Balster *et al.*, 1982; Moser *et al.*, 1985). Although the effect of TRI on the operant behavior of rats has not been previously

reported, the minimum effective concentration obtained in the present study is similar to those reported in rats to elicit changes in flash-evoked potentials and electroencephalograms (ATSDR, 1994), and well below levels required for unconditioned reflex failure, increased motor activity and ataxia (Clark and Tinston, 1982; Mullin and Krivanek, 1982; ATSDR, 1994).

In the present study, both changes in operant behavior and blood and brain concentrations of TRI were functions of the degree and duration of exposure. As expected for a lipid soluble chemical with a relatively low blood:air partition coefficient, the absorption pattern of TRI was characterized by very rapid uptake, with a rapid approach to steady state. Data from experiments in animals and humans provide supporting evidence that TRI is rapidly absorbed by the respiratory system. Dallas *et al.* (1989) have reported that arterial blood levels of TRI were quite high in rats within 2 minutes of exposure to 50 or 500 ppm. Furthermore, TRI was detected in the arterial blood of men within 10 seconds of exposure to 250 or 350 ppm (Astrand *et al.*, 1973). Once absorbed, TRI distributes among the various organs in proportion to organ blood flow and organ lipid content (Baker and Fine, 1986). As a result, TRI rapidly and extensively accumulates in the lipid-rich rat brain which is estimated to receive 2.21% of cardiac output while composing only 0.6% of total body weight (Dallas *et al.*, 1994).

Unfortunately, it cannot be assumed that animals and humans will react similarly upon inhaling the same TRI concentration since physiological differences exist among species that ultimately influence the amount and time-course of TRI deposition in the brain. For example, mice and rats have higher TRI blood:air partition coefficients than humans, and thus experience greater systemic uptake of TRI (Reitz *et al.*, 1988). Mice and rats also have higher respiratory and circulatory rates, two additional factors that contribute to a greater body burden. The impact of these physiological differences on TRI kinetics can be dramatic (Schumann *et al.*, 1982). For example, when normalized for differences in exposure concentration, the blood TRI levels of mice and rats reported

by Schumann *et al.* (1982) were 17.3 and 3.5 times those reported in humans by Nolan *et al.* (1984). The extrapolation of behavioral dose-response data generated in rodents, in the absence of comparative pharmacokinetic analysis, may therefore overstate human risk. As a result, it has been suggested that a scientifically-defensible approach to making interspecies extrapolations would assume that a particular target tissue dose in one species is equally as toxic in another (Andersen, 1987). Such an approach would benefit greatly from dose-response relationships where brain dose or a suitable dose surrogate is correlated with behavioral changes.

Because blood and brain concentrations in the present study were strongly correlated and equally predictive of operant performance, the blood TRI concentration would appear to be a suitable surrogate for brain concentration, provided sufficient time has elapsed for blood and brain concentrations to equilibrate. This is supported by the findings of Ameno *et al.* (1992) who showed strong correlations and linear relationships between blood and regional brain concentrations of toluene in rats. In addition, Bruckner and Peterson (1981) have concluded that blood concentration is a reasonable index of the depth of toluene-induced narcosis in mice as measured in tests of reflexes and unconditioned performance. In the present study, blood and brain concentrations of TRI were selected as dose metrics since 1) the very limited biotransformation of TRI makes metabolites of little concern to the CNS; 2) TRI's behavioral effects are thought to reflect the consequences of neuronal membrane fluidization, the degree of which is proportional to the amount of TRI dissolved therein; and 3) brain concentrations of TRI appear to be directly dependent on blood concentrations.

There appear to be important limitations, however, to the use of blood and brain concentrations as dose metrics in the present study. For example, after 60 minutes of exposure to 2000 ppm TRI, blood and brain concentrations equaled or exceeded those after 10 minutes of exposure to 5000 ppm. However, the 2000 ppm exposure group was unaffected 60 minutes into exposure, while the 5000 ppm exposure group exhibited a

drastic decline in response rate shortly after exposure initiation. This indicates that response rate suppression is not solely related to blood and brain concentrations, but may also be dependent upon the rate of TRI uptake. The rate of uptake has previously been implicated as a factor in m-xylene-induced body sway (Riihimaki and Savolainen, 1980) and diazepam-induced impairment of psychomotor skills (Linnoila and Mattila, 1973).

Insight into the species generality of the relationship between brain concentration and operant performance can be gained by examining the results of a parallel study to the present one, which employed CD-1 mice and a VI-60 reinforcement schedule (You *et al.*, 1994). Blood concentrations of TRI in mice were 2- to 3-fold higher than in rats during exposure to 3500 and 5000 ppm, yet brain concentrations were similar. This species difference in the brain:blood ratio of TRI is in general agreement with the richly perfused brain:blood partition coefficients estimated for rats (1.49) and mice (0.796) (Reitz *et al.*, 1987). Therefore, despite differences in the blood concentration of TRI, similar brain concentrations in mice and rats might be expected to result in similar effects on operant performance. The threshold concentration for response rate decreases in the present study is 35 μg/g brain. However, mice exposed to 3500 ppm for 100 minutes exhibited no decline in VI-60 responding despite an end-exposure brain concentration of 132 μg/g. At 5000 ppm, a response rate decrease in mice was not evident until 20 minutes of exposure, when the brain concentration was $\geq$ 67 μg/g. These results suggest that mice may be inherently less sensitive than rats to operant disruption by TRI while responding on a VI schedule.

In another investigation, Balster *et al.* (1982) reported a 25% reduction in fixed ratio responding of mice exposed to 2000 ppm TRI for 20 minutes. Based on results of the present study, a 25% decline in response rate would be expected to occur at a brain concentration of 60.8 μg/g. Interestingly, pharmacokinetic data (Holmberg *et al.*, 1977), once normalized for changes in exposure concentration, indicate that the brain concentration of TRI in mice after such an exposure would nearly equal the expected

value. However, since the schedule of reinforcement influences the sensitivity of operant behavior to toxicants, such a comparison may be somewhat misleading.

The effects of TRI on human behavior are also interesting in light of the present study. Mackay *et al.* (1987) exposed human volunteers to 175 and 350 ppm TRI for 3.5 hours, during which psychomotor tests were periodically administered and blood withdrawn for analysis. Deficits in tracking skill and reaction time were first observed at blood concentrations ($\approx$ 0.50 and 1.60 μg/ml) well below the threshold blood level for response rate decreases in the present study (15 μg/ml), which can only be explained in small part by the differing richly perfused brain:blood partition coefficients reported for humans (3.40) and rats (1.49) (Reitz *et al.*, 1987). In addition, when steady-state blood levels measured in humans exposed to 350 ppm (Mackay *et al.*, 1987; Nolan *et al.*, 1984) are multiplied by the estimated richly perfused brain:blood partition coefficient, estimated brain concentrations range from 5.96-10.88 μg/g. These values are 17-31% of the threshold brain concentration necessary for response rate suppression in the rat, yet are sufficient to elicit psychomotor impairment in humans (Mackay *et al.*, 1987). Tracking skill and reaction time in humans therefore, appear to be far more sensitive to the effects of TRI than the well conditioned and highly motivated operant responding of rats. Stewart *et al.* (1961) have published results that suggest the same may be true for lightheadedness in humans.

Unfortunately, the present effort represents one of only a few attempts to correlate the pharmacokinetics of TRI with CNS pharmacodynamics. Indeed, few studies with any solvent, with the exception of alcohol, have had this goal in mind. Among these is a study by Riihimaki and Savolainen (1980) in which impaired body balance in volunteers was not only correlated with blood m-xylene concentrations, but was also shown to depend on a rapid rise of the blood m-xylene level. In addition, Bruckner and Peterson (1981) have shown that the magnitude of toluene-induced impairment of reflexes and unconditioned performance in mice is paralleled by blood and brain concentrations of

toluene. More recently, Kishi *et al.* (1993) have reported a relationship between blood trichloroethylene levels and shock avoidance performance decrements in rats. It is clear that due to ethical considerations, further progress in understanding the risk that solvents pose to the human CNS rests with performing animal studies. It is therefore imperative that the most sensitive behavioral tests be used in conjunction with physiologically and anatomically well characterized animals to create a data base to which state-of-the-art extrapolation methods can be applied.

The relationship between blood and brain concentrations of solvents and behavior is obviously complex. Such potential confounding factors as acute neuronal adaptation, biphasic response patterns, and uncertainty as to the neurological basis of many behaviors contribute to this complexity and will clearly make these relationships more difficult to determine. In addition, solvents have been shown to differentially distribute in the brain on the basis of regional lipid content (Gospe and Calaban, 1988; Ameno *et al.*, 1992) and, therefore, considering the CNS as a single homogeneous compartment for kinetic purposes or as a dose metric may be inappropriate. However, as pointed out by Weiss (1988), pharmacokinetic models deserve to be accompanied by behavioral endpoints of at least equal quantitative stature. Lack of knowledge of the relationship between blood or brain concentration and altered behavior would render such models inappropriate for interspecies extrapolation. Thus, the study described herein represents an initial effort to utilize behavioral modifications as an acceptable toxicological endpoint for risk assessment.

REFERENCES

Ameno, K., Kiriu, T., Fuke, C., Ameno, S., Shinohara, T., and Ijiri, I. (1992). Regional brain distribution of toluene in rats and in human autopsy. *Arch. Toxicol.* 66, 153-156.

Andersen, M.E. (1987). "Tissue dosimetry in risk assessment, or what's the problem here anyway?" In *Drinking Water and Health*, Vol.8, pp. 8-26. National Academy Press, Washington, DC.

Astrand, L., Kilbom, A., Wahlberg, I., and Ovrum, P. (1973). Methylchloroform exposure. I. Concentration in alveolar air and blood at rest and during exercise. *Work Environ. Health.* 10, 69-81.

(ATSDR) Agency for Toxic Substances and Disease Registry. (1994). *Toxicological Profile for 1,1,1-Trichloroethane.* U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Baker, E.L., and Fine, L.J. (1986). Solvent neurotoxicity: the current evidence. *J. Occup. Med.* 28(2), 126-129.

Balster, R.L., Moser, V.C., and Woolverton, W.L. (1982). Concurrent measurements of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J. Pharmacol. Methods.* 8, 299-309.

Bruckner, J.V., and Peterson, R.G. (1981). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol. Appl. Pharmacol.* 61, 27-38.

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. (1993). Analyses of volatile C_2 haloethanes and haloethenes in tissues: sample preparation and extraction. *J. Chromatogr.* 612, 199-208.

Clark, D.G., and Tinston, D.J. (1982). Acute inhalation toxicity of some halogenated and nonhalogenated hydrocarbons. *Hum. Toxicol.* 1, 239-247.

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M., and Bruckner, J.V. (1994). Use of a physiologically based model to predict systemic uptake and respiratory elimination of perchloroethylene. *Toxicol. Appl. Pharmacol.* 128, 60-68.

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. (1989). The uptake and elimination of 1,1,1-trichloroethane during and following inhalation exposures in rats. *Toxicol. Appl. Pharmacol.* 98, 385-397.

Evans, E.B., and Balster, R.L. (1991). CNS depressant effects of volatile organic solvents. *Neurosci. Biobehav. Rev.* 15, 233-241.

Gad, S., and Weil, C.S. (1986). *Statistics and Experimental Design for Toxicologists.* Telford Press, Caldwell, New Jersey.

Gamberale, F., and Hultengren, M. (1973). Methylchloroform exposure II. Psychophysiological functions. *Scand. J. Work Environ. Health.* 10, 82-92.

Geller, I., Mendez, V., Hartmann, R.J., Gause, E., and Rippstein, W.J. (1982). Effects of 1,1,1-trichloroethane on a match-to-sample discrimination task in the baboon. *J. Tox. Env. Health.* 9, 783-795.

Gospe, S.M., and Calaban, M.J. (1988). Central nervous system distribution of inhaled toluene. *Fund. Appl. Toxicol.* 11, 540-545.

Holmberg, B., Jakobson, I., and Sigvardsson, K. (1977). A study on the distribution of methylchloroform and n-octane in the mouse during and after inhalation. *Scand. J. Work Environ. Health.* 3, 43-52.

Hurst, P.M., and Bagley, S.K. (1972). Acute adaptation to the effects of alcohol. *Quart. J. Stud. Alc.* 33, 358-378.

Huynh, H., and Feldt, L.S. (1976). Estimation of the Box correction for degrees of freedom from sample data in the randomized block and split-plot designs. *J. Educ. Stat.* 1, 69-82.

Jones, B.M., and Vega, A. (1972). Cognitive performance measured on the ascending and descending limb of the blood alcohol curve. *Psychopharmacologia.* 23, 99-114.

Kishi, R., Harabuchi, I., Ikeda, T., Katakura, Y., and Miyake, H. (1993). Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans. *Br. J. Ind. Med.* 50, 470-480.

Linnoila, M., and Mattila, M.J. (1973). Drug interaction on psychomotor skills related to driving: Diazepam and alcohol. *Eur. J. Pharmacol.* 5, 186-194.

Mackay, C.J., Campbell, L., Samuel, A.M., Alderman, K.J., Idzikowski, C., Wilson, H.K., and Gompertz, D. (1987). Behavioral changes during exposure to 1,1,1-trichloroethane: Time-Course and relationship to blood solvent levels. *Am. J. Ind. Med.* 11, 223-239.

Moser, V.C., and Balster, R.L. (1986). The effects of inhaled toluene, halothane, 1,1,1-trichloroethane, and ethanol on fixed-interval responding in mice. *Neurobehav. Toxicol. Teratol.* 8, 525-531.

Moser, V.C., Scimeca, J.A., and Balster, R.L. (1985). Minimal tolerance to the effects of 1,1,1-trichloroethane on fixed-ratio responding in mice. *Neurotoxicology.* 6(1), 35-42.

Mullin, L.S., and Krivanek, N.D. (1982). Comparison of unconditioned reflex and conditioned avoidance tests in rats exposed by inhalation to carbon monoxide, 1,1,1-trichloroethane, toluene or ethanol. *NeuroTox.* 3, 126-137.

Nolan, R.J., Freshour, N.L., Rick, D.L., McCarty, L.P., and Saunders, J.H. (1984). Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. *Fund. Appl. Toxicol.* 4, 654-662.

Orr, J., Dussault, P., Chappel, C., Goldberg, L., and Reggiani, G. (1976). Relation between drug-induced central nervous system effects and plasma levels of diazepam in man. *Mod. Probl. Pharmacopsych.* 11, 57-67.

Radlow, R., and Hurst, P.M. (1985). Temporal relations between blood alcohol concentration and alcohol effect: an experiment with human subjects. *Psychopharmacology*. 85, 260-266.

Reitz, R.H., McDougal, J.N., Himmelstein, M.W., Nolan, R.J., and Schumann, A.M. (1988). Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose, and dose route extrapolations. *Toxicol. Appl. Pharmacol.* 95, 185-199.

Reitz, R.H., Nolan, R.J., and Schumann, A.M. (1987). "Development of multispecies, multiroute pharmacokinetic models for methylene chloride and 1,1,1-trichloroethane (methyl chloroform)." In *Drinking Water and Health*, Vol.8, pp. 8-26. National Academy Press, Washington, DC.

Riihimaki, V., and Savolainen, K. (1980). Human exposure to m-xylene. Kinetics and acute effects on the central nervous system. *Ann. Occup. Hyg.* 23, 411-422.

Rowland, M., and Tozer, T.N. (1980). Appendix B. Assessment of Area. In: *Clinical Pharmacokinetics: Concepts and Applications*. Lea and Febiger, Philadelphia, pp. 288-291.

Schumann, A.M., Fox, T.R., and Watanabe, P.G. (1982). [^{14}C]Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. *Toxicol. Appl. Pharmacol.* 62, 390-401.

Sidell, F.R., and Pless, J.E. (1971). Ethyl alcohol: Blood levels and performance decrements after oral administration to man. *Psychopharmacologia.* 19, 246-261.

Stewart, R.D., Gay, H.H., Erley, D.S., Hake, C.L., and Schaffer, A.W. (1961). Human exposure to 1,1,1-trichloroethane vapour: relationship of expired air and blood concentrations. *Am. Ind. Hyg. Assoc. J.* 22, 252-262.

Torkelson, T.R., Oyen, F., McCollister, D.D., and Rowe, V.K. (1958). Toxicity of 1,1,1-trichloroethane as determined on laboratory animals and human subjects. *Am. Ind. Hyg. Assoc. J.* 19, 353-362.

Wallenstein, S., Zucker, C.L., and Fleiss, J. (1980). Some statistical methods useful in circulation research. *Circ. Res.* 47, no.1, 1-9.

Weiss, B. (1988). Quantitative perspectives on behavioral toxicology. *Toxicol. Lett.* 43, 285-293.

Winer, B.J. (1971). *Statistical Principles in Experimental Design.* 2nd ed., McGraw, New York.

You, L., Muralidhara, S., and Dallas, C.E. (1994). Comparisons between operant response and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain. *Toxicology.* 93, 151-163.

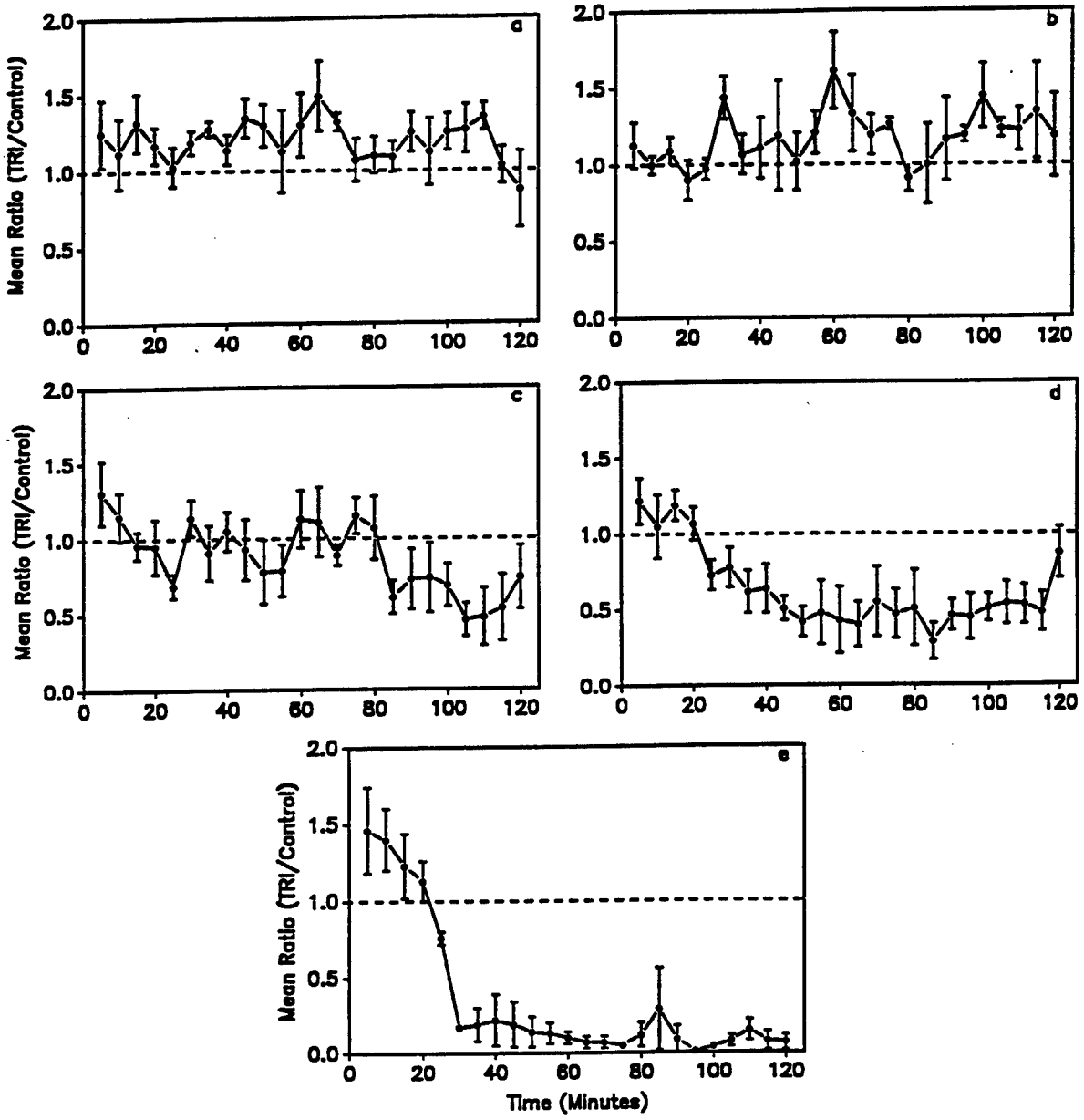

Figure 1: Operant responding during the 20 minutes preceding and 100 minutes following the onset of continuous exposure to a) 500, b) 1000, c) 2000, d) 3500 and e) 5000 ppm TRI. Each data point represents the mean operant response ratio (TRI/Control) ± SE of five rats during each 5-minute interval of 120-minute operant sessions.

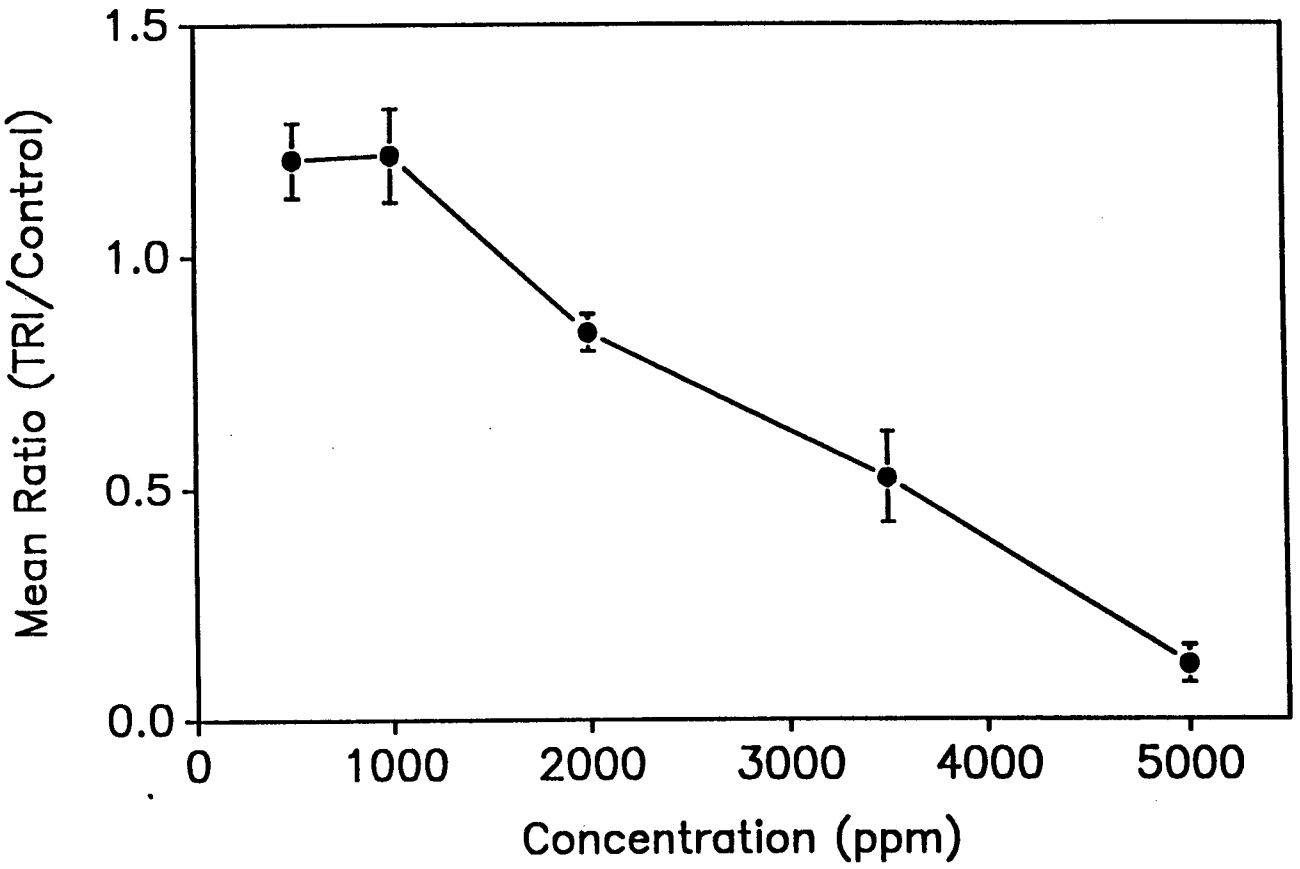

Figure 2: Dose-response curve relating operant responding to exposure concentration of TRI. Each data point represents the average ± SE of mean operant response ratios (TRI/Control) during 100-minute exposures to 500, 1000, 2000, 3500 and 5000 ppm TRI.

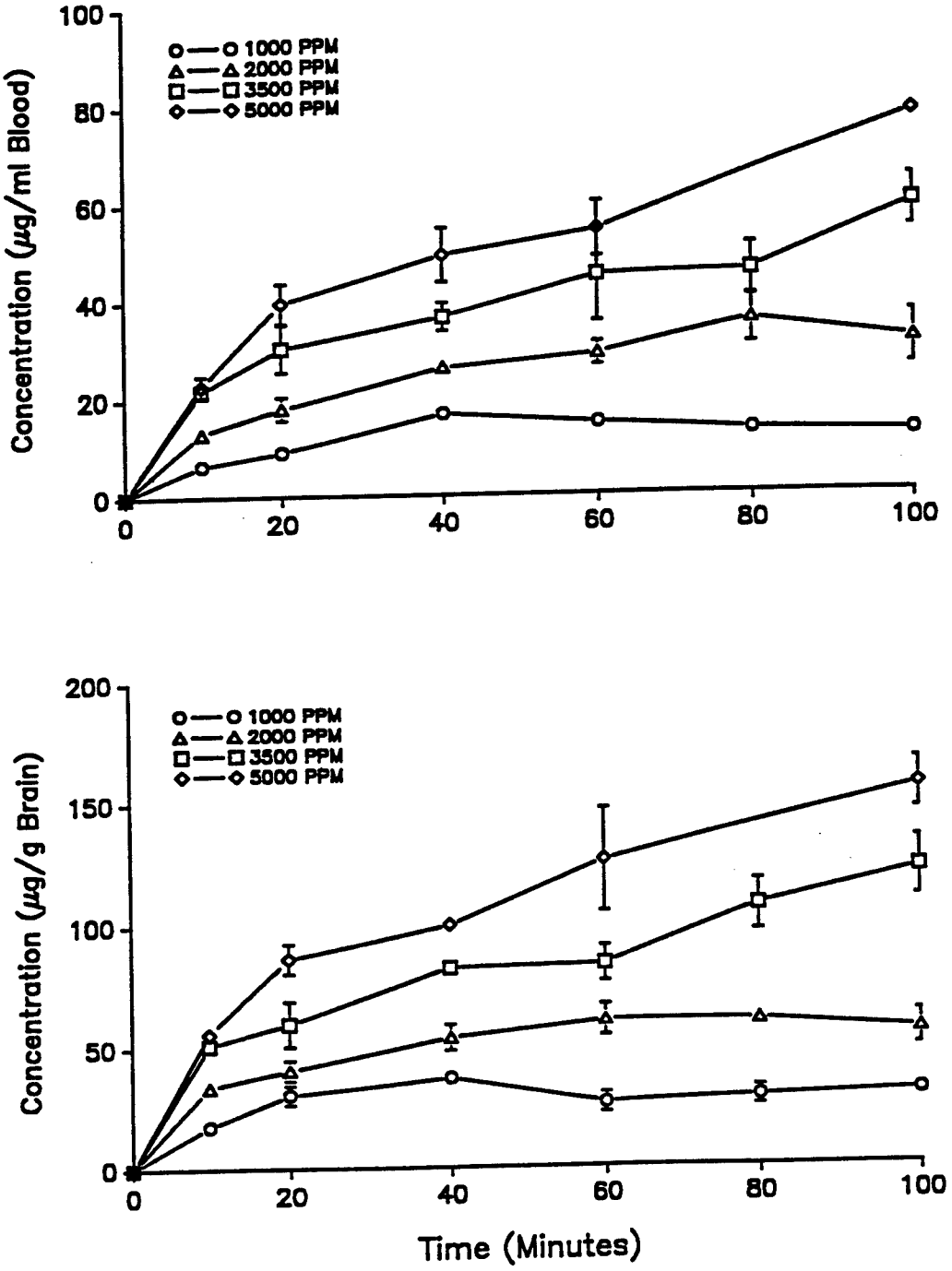

Figure 3: Uptake of TRI in a) blood and b) brain during 100-minute exposures to 1000, 2000, 3500 and 5000 ppm TRI. Each data point represents the mean ± SE of five rats. SE bars are obscured by symbols in some cases.

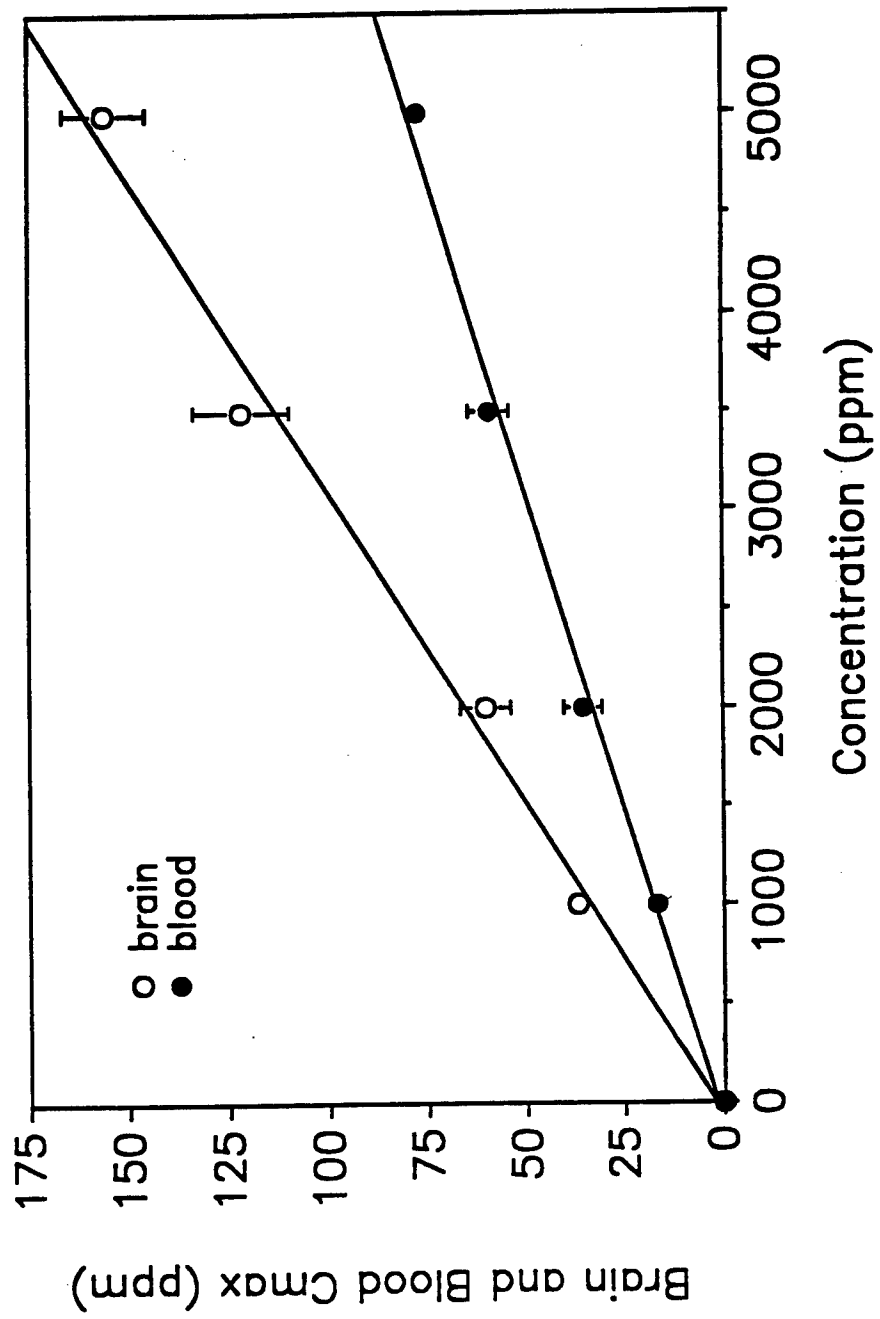

Figure 4: Maximum blood and brain concentrations during 100-minute exposures to TRI, as a function of inhaled concentration. Each data point represents the mean ± SE of five rats.

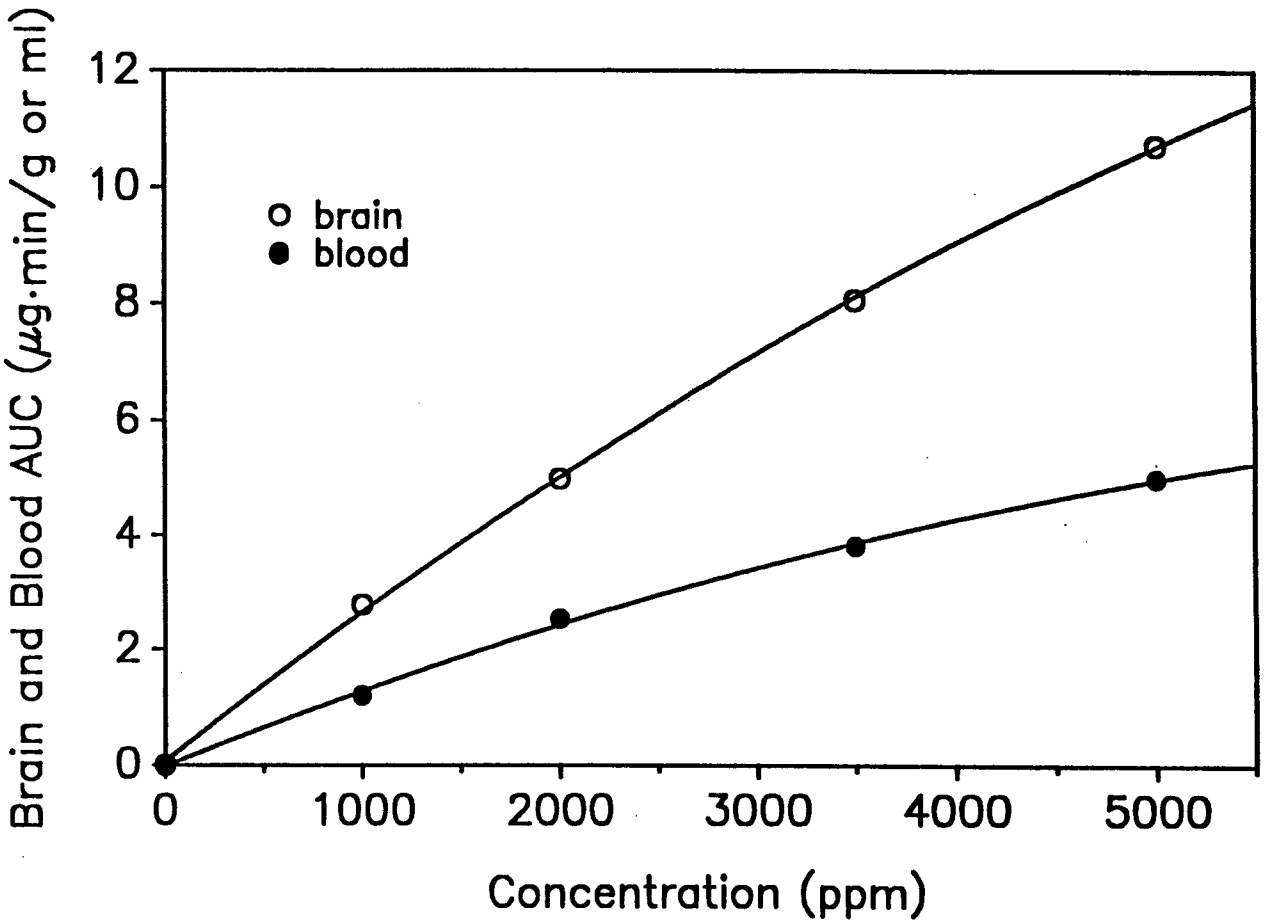

Figure 5: Areas-under-the-blood- and brain-concentration versus time curves presented in Figure 3, as a function of inhaled concentration.

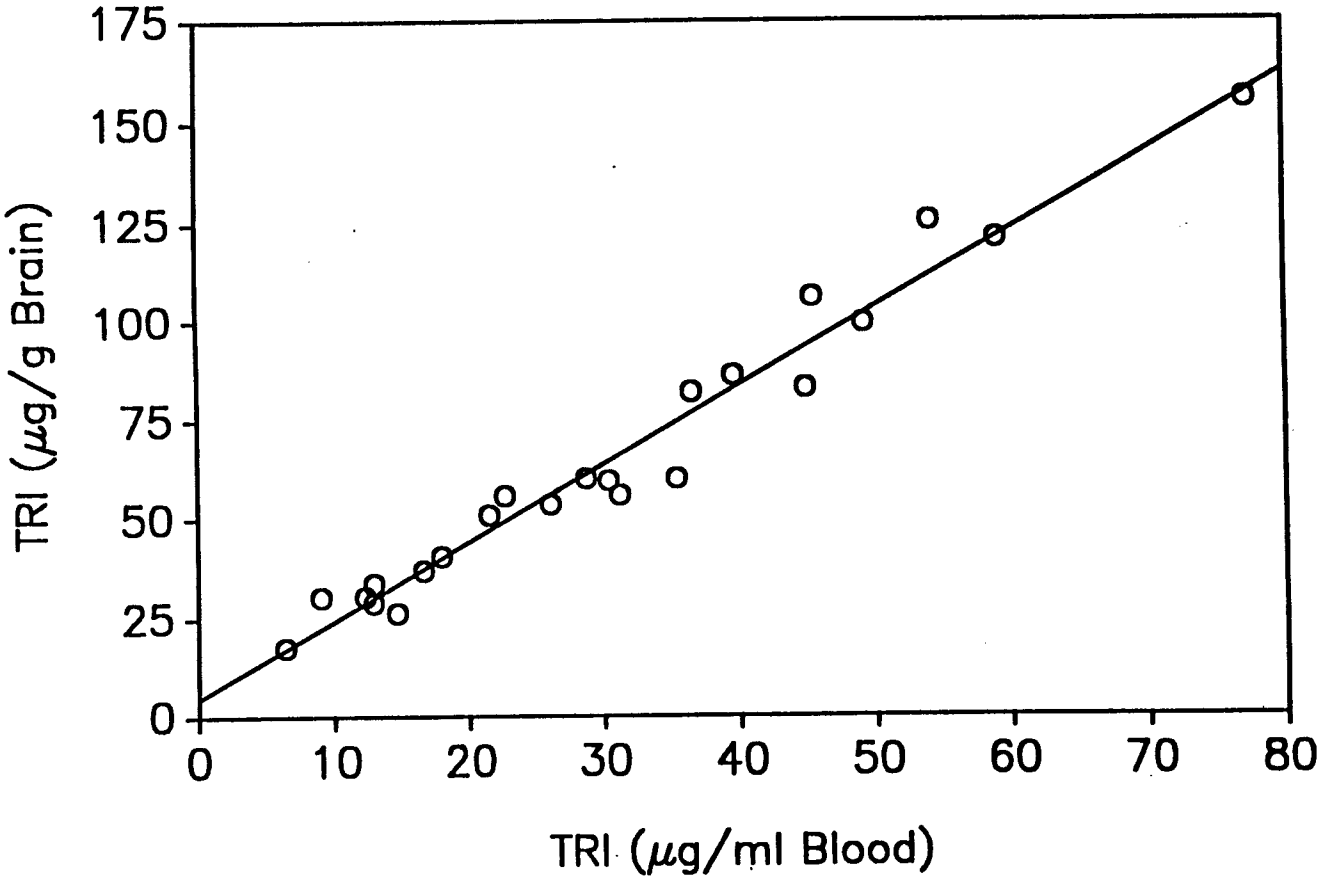

Figure 6: Scatter plot relating blood and brain concentrations of TRI. Each data point represents the mean blood and brain concentration of five rats after 10, 20, 40, 60, 80 or 100 minutes of exposure to 1000, 2000, 3500 or 5000 ppm TRI. The equation of the regression line is $y = 1.977x + 4.587$.

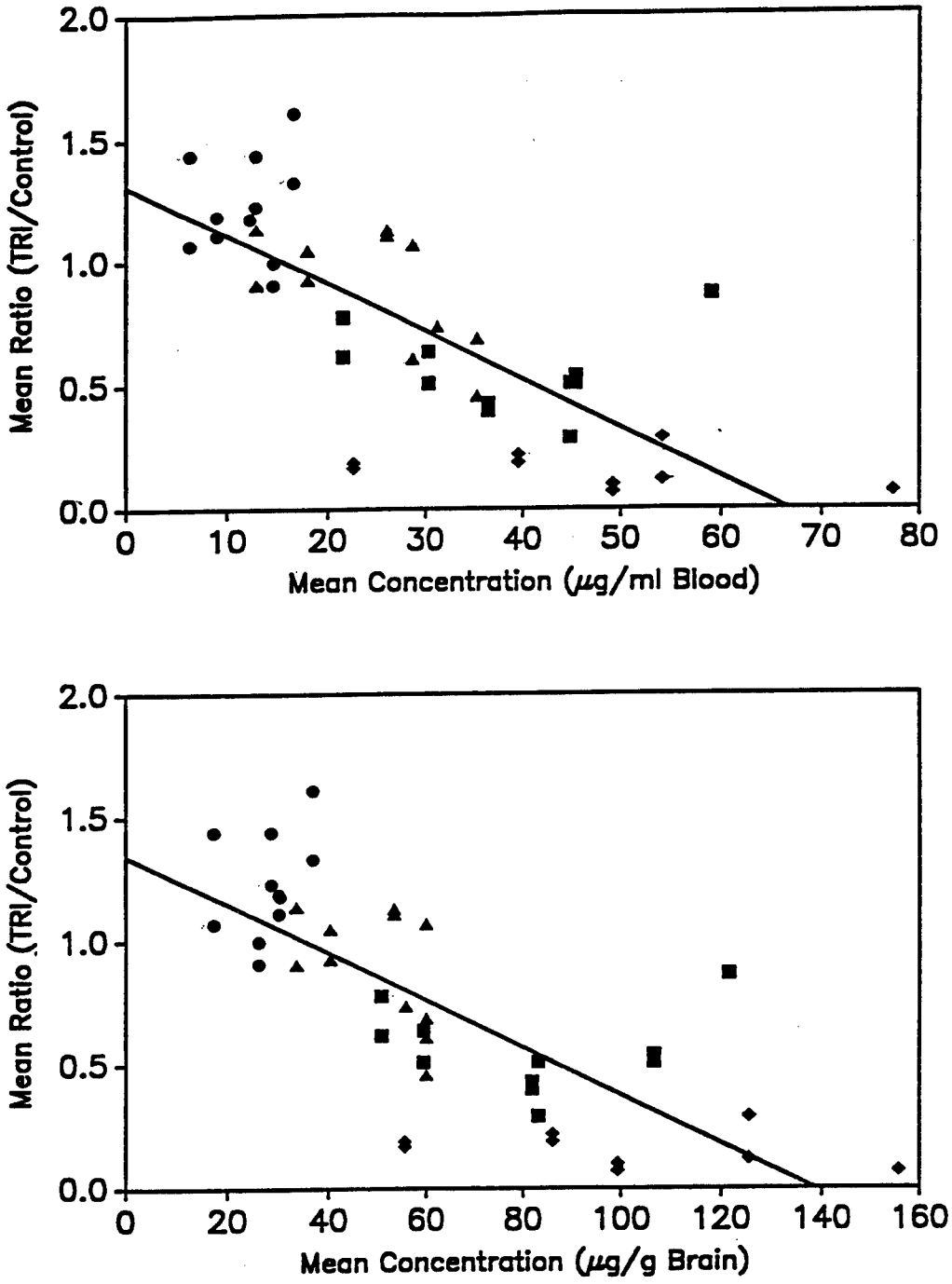

Figure 7: Scatter plots relating operant responding to a) blood and b) brain concentrations of TRI. Each data point represents the mean blood or brain concentration of five rats after 10, 20, 40, 60, 80 or 100 minutes of exposure to 1000 (•), 2000 (▲), 3500 (■) or 5000 ppm TRI (◆), as well as the mean operant response ratio (TRI/Control) of five rats during the 5-minute intervals immediately preceding and following blood and brain collection. The equations of the regression lines are $y = -0.019x + 1.29$ (blood) and $y = -0.0097x + 1.34$ (brain).

DOSE-RESPONSE CURVES FOR THE EFFECT OF 1,1,1-TRICHLOROETHANE ON THE OPERANT BEHAVIOR OF SINGLY AND REPEATEDLY EXPOSED RATS[1]

[1]Warren, D.A., Reigle, T.G., and Dallas, C.E. To be submitted to *Journal of the American College of Toxicology*.

ABSTRACT

A design feature of most dose-response studies involving schedule-controlled operant behavior is the repeated administration of different doses of the test substance to the same experimental animal. Repeated dosing raises the question of whether or not an animal's initial exposure to a chemical agent alters its behavioral response to subsequent exposures. To address this question, a dose-response curve for the effect of inhaled 1,1,1-trichloroethane (TRI) on the rate of lever-pressing for milk delivery was generated with repeatedly exposed rats (i.e., a within-subject design) and compared to dose-response data obtained from rats receiving a single inhalation exposure to TRI (i.e., a between-group design). Relative to that generated with singly exposed rats, the dose-response curve generated by repeated exposure was shifted to the left. This suggests that the behavioral effects of rate-decreasing concentrations of TRI are augmented by previous exposures. This residual effect is apparently not due to the accumulation of pharmacologically active substances or to the development of an aversion to responding, since TRI is rapidly eliminated following exposure and solvent-free responding was unaffected 24 hours post exposure. Instead, the results of this study support the well established belief that an animal's response to a drug or chemical agent can be modified by its prior behavioral and exposure history. Thus, comparisons of single and repeated exposures are essential for fully accurate interpretations of the behavioral consequences of solvent exposure.

INTRODUCTION

Inhalation of volatile organic solvents occurs in the workplace and as a form of drug abuse (Balster, 1987). Such solvents are capable of producing a general depression of the central nervous system (CNS), with consequent impairment of cognition and motor

skills in a concentration-dependent manner. In recent years, there has been an increased interest in the behavioral effects of these agents with a resultant increase in the study of their effects on schedule-controlled operant behavior (SCOB). Changes in operant behavior are thought to reflect effects of solvents in laboratory animals which are comparable to psychomotor changes in humans (ATSDR, 1994). As a result, several reports have described the rate-increasing and rate-decreasing effects of solvents on steady-state responding in rodents (Glowa, 1985; Evans and Balster, 1991). In some cases, the relationship between inhaled concentration and effect, i.e., the dose-response curve, has been determined. As behavior is increasingly being considered a potential regulatory endpoint, the need for dose-response determinations will likely increase in order to provide a basis for the development of rational limits for human exposure.

In view of the large number of solvents in use today and the continual introduction of new agents, rapid and inexpensive methods will be needed to assess their behavioral toxicity (Glowa *et al.*, 1983). Methods involving learned behaviors in general, and SCOB in particular, have often been deemed too time consuming and labor intensive for routine use (Wenger, 1990). However, unlike many of the more recently introduced behavioral tests, previous studies of SCOB have provided an extensive background of information against which the effects of solvents may be compared (Rice, 1988). In addition, simpler procedures such as the measurement of unconditioned reflexes and motor activity reflect different aspects of behavior and are often less sensitive measures of solvent exposure (Sette and Levine, 1986; Claudio, 1992). Therefore, the economical use of SCOB would be of considerable benefit to the study of behavioral toxicology.

One economical design feature common to most dose-response studies involving SCOB is the repeated administration of different doses to the same animal. Indeed, the practicality of SCOB largely depends on within-subject designs due primarily to the laborious task of training animals to some level of behavioral stability. In turn, the

appropriateness of some within-subject designs depends upon whether chemical exposure irreversibly changes an animal's behavior in such a manner that would be reflected in its behavioral reaction to subsequent exposures (Sidman, 1960). If irreversible changes occur, the resulting dose-response data would not be a pure function of concentration, but would also be a function of the consequences of previous exposure (Sidman, 1960).

The generation of cumulative dose-response curves by exposing animals to incrementally increasing solvent concentrations in a single operant session is a widely accepted practice in behavioral toxicology (Glowa *et al.*, 1983; Glowa, 1991; Glowa, 1993). With such a design, the potential for solvent accumulation is great, and exposure to early solvent concentrations might be expected to influence the behavioral reaction to subsequent exposures. Because solvents classically exhibit a rapid rate of elimination, an alternative means of generating dose-response curves using a within-subject design would be to allow sufficient time between exposures for complete solvent clearance. The question remains, however, whether or not solvent exposure in the absence of solvent accumulation, would also influence an animal's response to subsequent exposures. To address this question, dose-response curves for the effect of 1,1,1-trichloroethane (TRI) on the rate of lever-pressing for milk delivery were generated using both singly and repeatedly exposed rats. Similar dose-response curves would suggest that prior exposure has little or no influence on operant responding, whereas differing dose-response functions would suggest that previous TRI exposures exerted a residual effect. 1,1,1-Trichloroethane was selected for study because it has a short half-life, undergoes minimal metabolism, and has relatively well characterized effects on SCOB. Using this solvent, it was believed that the results of the current investigation would provide an enhanced understanding of the factors which influence animal behavior and would enable a more accurate interpretation of the behavioral consequences of solvent exposure.

MATERIALS AND METHODS

Chemical: 1,1,1-Trichloroethane of 97%+ purity was obtained form Aldrich Chemical Co., Inc. (Milwaukee, WI).

Animals: Male Sprague-Dawley rats (275-350 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were housed two per cage in suspended wire-bottom cages (36 x 20 x 20 cm) in a temperature- (22°C) and humidity- (45%) controlled room with a 12-hour light-dark cycle (light: 0700-1900 hr). Rats were acclimated for at least 7 days prior to use, during which time food (Purina Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*. All experiments were conducted during the light phase of the light-dark cycle.

Behavioral and Exposure Apparatus: Operant sessions were conducted in a slotted test cage (Coulbourn Instruments, Inc., Lehigh Valley, PA) equipped with a house light, response lever, liquid delivery trough and dipper, and a stimulus light above the delivery trough that remained lit during the availability of the milk reinforcer. The test cage was interfaced via LabLinc (Coulbourn Instruments, Inc., Lehigh Valley, PA) with an IBM-compatible 386 computer running COSMOS software (Coulbourn Instruments, Inc., Lehigh Valley, PA) that applied the operant performance schedule and recorded the number of responses and reinforcers in each 5-minute interval of the operant session. The test cage was positioned inside a 1.0 M^3 Rochester-type dynamic flow inhalation chamber that served not only to expose the animals, but also to isolate them from extraneous stimuli. Nitrogen was passed through a glass dispersion flask of liquid TRI from which solvent vapor entered the chamber's influent air stream. A heating mantle was placed around the dispersion flask to generate vapor concentrations $\geq$ 2000 ppm. The flask was enclosed in a plexiglass safety box under constant negative pressure. Exhaust air from the inhalation chamber and safety box was vented through HEPA and activated charcoal filters prior to its release into the environment. Vapor concentrations

were continuously monitored with a Miran 1B2 portable infrared spectrophotometer (λ = 9.4 μm) (The Foxboro Co., East Bridgewater, MA) interfaced with a microcomputer-based Foxboro DL 332F Datalogger (Metrosonics Inc., Rochester, NY). The Miran was calibrated with a closed loop system (The Foxboro Co., East Bridgewater, MA) and the calibration accuracy verified just prior to each exposure with liquid TRI injections that volatilized to produce concentrations spanning the calibration range. Target vapor concentrations were reached within 2-5 minutes and thereafter exhibited $\pm$ 5% random fluctuation. Occasional adjustments in nitrogen flow and heating mantle temperature were necessary to maintain target concentrations.

Operant Behavior: Rats were transferred to individual polypropylene cages (48 x 25 x 20 cm) with corn cob bedding and stainless steel wire lids following acclimation. The animals were food-restricted (10 $\pm$ 0.25 g/day) during a period in which they were trained to lever-press for the presentation of undiluted evaporated milk (0.08 ml for 7 seconds/reinforcement) on a variable interval-30 second (VI-30) reinforcement schedule (i.e., a lever-press produced milk every half minute on average). Initially, rats were manually reinforced in 30-minute sessions for coming near the lever or inadvertently touching it. Once rats learned to respond independently, the mean interval between reinforcer availability was gradually increased to 30 seconds and the session length extended to 120 minutes. Rats responded in daily 120-minute sessions spaced 24 hours apart until their response rates stabilized, a process requiring 15-20 days. The criterion for stable behavior was four successive sessions in which the number of responses/second varied by less than 15% from the 4-day mean rate and showed no significant time trend. Once rats met the stability criterion, their behavior was monitored during exposure to clean air for 20 minutes, followed by a single concentration of TRI vapor (500, 1000, 2000, 3500 or 5000 ppm) for 100 minutes. Five chemically-naive rats were exposed to each TRI concentration.

The same methodology was followed in the preparation of animals for repeated TRI exposure. However, during efforts to establish stable baseline response rates, several rats exhibited reduced rates of lever-pressing in the terminal half of their operant training sessions. Thus, the reinforcement schedule was shifted from a VI-30 to a VI-60 (a lever-press produced milk every minute on average) in order to reduce the reinforcement density. Once rats responding on a VI-60 schedule met the stability criterion ($n = 9$), four rats were repeatedly exposed to TRI in the order 1000, 5000, 3000, 4000 and 2000 ppm (Group A), and the remaining five rats in the order 4000, 1000, 5000, 3000 and 2000 ppm (Group B). Exposures were separated by 48 hours, and 24 hours after each exposure, rats responded for 120 minutes in a solvent-free atmosphere.

Data Analysis: The control behavioral response of each singly and repeatedly exposed animal was calculated as the average number of lever-presses during the last 100 minutes of the four operant sessions used to meet the stability criterion. Time trends in responding were examined by linear regression analysis (Wallenstein *et al.*, 1980). The number of responses during the last 100 minutes of the solvent-free operant sessions that were conducted 24 hours after each exposure were reported as a proportion of control. The effect of each TRI concentration in individual rats was determined by calculating the ratio of the rate of responding in the presence of TRI to the control rate of responding (TRI/Control). Dose-response data are presented as the mean $\pm$ standard error (SE) of these individual determinations. Additionally, the operant response ratios of the nine repeatedly exposed animals were plotted in comparison to the dose-response curve generated with singly exposed animals. Response ratios at 3000 and 4000 ppm were estimated for singly exposed animals from a least squares linear regression line ($y = -0.000253x + 1.405$; $r^2 = 0.99$) that was based on effects observed at 1000, 2000, 3500 and 5000 ppm. Student's t-tests were used to compare dose-response data of singly and repeatedly exposed rats with a minimum level of significance of $p \leq 0.05$. The EC_{50} value (concentration of TRI expected to decrease responding by 50%) was determined

for singly exposed animals by solving the equation for the least squares linear regression line where y = 0.5, and the confidence interval (CI) was defined using SAS (SAS Institute, Cary, NC). Measures of variation are standard deviations (SD) unless otherwise specified.

RESULTS

Rats responding under the variable interval schedule displayed a characteristic moderate, but steady rate of responding, with little or no pausing evident after each reinforcement (Weiss and Cory-Slechta, 1994). The control response rates of animals singly exposed to 500, 1000, 2000, 3500 and 5000 ppm TRI were 0.31 ± 0.15, 0.31 ± 0.17, 0.46 ± 0.10, 0.36 ± 0.08 and 0.44 ± 0.11 responses/second, respectively. The mean (± SD) control response rate of repeatedly exposed animals was 0.43 ± 0.25 responses/second, with a range from 0.15 to 0.76 responses/second for individual animals. Therefore, the use of slightly different variable interval schedules (VI-30 versus VI-60) did not result in response rate differences sufficient to account for any differential response to TRI between repeatedly and singly exposed animals. Neither the singly nor repeatedly exposed animals exhibited a significant time trend in the rate of responding during control or solvent-free operant sessions. Repeated exposures to TRI did not appear to affect solvent-free responding, as response rates returned to control levels when examined 24 hours post exposure (Figure 1). Such recovery occurred following all conditions of TRI exposure, including concentrations which nearly caused the complete cessation of responding 24 hours earlier.

A comparison of the dose-response curves generated by exposing groups of rats to a single TRI concentration and individual rats to multiple TRI concentrations is shown in Figure 2. Response ratios of singly exposed animals averaged 1.20 ± 0.15, 1.20 ± 0.17, 0.83 ± 0.22, 0.53 ± 0.14 and 0.15 ± 0.16 for the 500, 1000, 2000, 3500 and

5000 ppm groups, respectively. For repeatedly exposed animals, response ratios during TRI exposure to 1000, 2000, 3000, 4000 and 5000 ppm averaged 1.17 ± 0.13, 0.45 ± 0.08, 0.18 ± 0.04, 0.45 ± 0.17 and 0.25 ± 0.07, respectively. Relative to that generated with singly exposed animals, the dose-response curve generated with repeatedly exposed animals was shifted to the left, indicative of a greater response to equivalent concentrations of TRI. Whereas the EC_{50} in singly exposed animals was 3577 ppm (95% CI = 2864-4253 ppm), average response rate reductions of 55 and 82% were produced in repeatedly exposed animals at 2000 and 3000 ppm, respectively. These drastic response rate reductions occurred immediately upon the initiation of exposure in the majority of animals.

Response ratios for animals singly exposed to 3000 and 4000 ppm were estimated to be 0.65 ± 0.06 and 0.39 ± 0.06, respectively. Student's t-tests detected highly significant differences in the response ratios of singly and repeatedly exposed animals at 2000 ($t = 4.25$, $df = 12$, $p < 0.0005$) and 3000 ppm ($t = 9.63$, $df = 12$, $p < 0.0005$). At these two concentrations, all nine repeatedly exposed animals exhibited response ratios at or below the average response ratio measured or estimated in singly exposed animals (Figure 3). The majority of repeatedly exposed animals also had lower response ratios at 1000, 4000 and 5000 ppm, although interanimal variability was high. Whereas the dose-response curve reflected increasing effects with increasing dose for singly exposed animals, this was not the case for repeatedly exposed animals. On average, rats in Groups A and B showed little or no difference in their responses to 2000, 3000, 4000 and 5000 ppm.

The statistically significant differences in response ratios of singly and repeatedly exposed animals at 2000 and 3000 ppm may have been due in part to the order of exposure concentrations. The lowest average response ratio for repeatedly exposed animals was at 3000 ppm, which immediately followed a concentration (5000 ppm) that had drastic rate-decreasing effects in all nine animals just 48 hours earlier. In addition,

2000 ppm was the fifth and final concentration for all nine repeatedly exposed animals, and therefore, exposure to three rate-decreasing concentrations had already occurred within the last 8 days.

DISCUSSION

Although the CNS is highly sensitive to the effects of many solvents, few studies have examined the dose dependency of solvent effects on steady-state behavior under the control of operant schedules. Balster *et al.* (1982) and Moser *et al.* (1985) have, however, demonstrated concentration-dependent decreases in response rates during TRI exposure in mice trained on fixed-ratio 100 reinforcement schedules. Both of these studies used repeated or cumulative exposure regimens for the determination of dose-response relationships and, although comparisons between these investigations and the current study are limited by the use of different species, exposure parameters and reinforcement schedules, comparable EC_{50} values were obtained. The two previous studies reported EC_{50} values of 2836 ppm (95% CI = 2042-3631 ppm) and 2727 ppm (95% CI = 1622-4365 ppm), versus an EC_{50} for singly exposed animals in the present study of 3577 ppm (95% CI = 2864-4253 ppm). However, results of the current investigation indicate that prior exposure to rate-decreasing concentrations of TRI augments the rate-decreasing effects of subsequent exposures, thereby altering the dose-response relationship. That VI-60 responding in the current study had recovered to control rates when measured 24 hours post exposure would indicate that such recovery is not synonymous with the complete reversibility of TRI's behavioral effects. Complete reversibility may only be necessary, however, when quantitative accuracy is a primary concern, as when such data are used in a risk assessment context. Indeed, the residual effect of rate-decreasing exposures may be beneficial for hazard identification purposes by increasing test sensitivity.

The residual effect of rate-decreasing TRI concentrations could be due to pharmacological factors, behavioral factors, or both (Liang *et al.*, 1983). That pharmacological factors are responsible is unlikely since there is little potential for TRI to accumulate during the exposure regimen employed in the current study. Schumann *et al.* (1982a) have reported, for example, that 96-99% of the total body burden of TRI in rats is eliminated 24 hours after a 6 hour inhalation exposure to 150 and 1500 ppm. Although higher concentrations were used in the present study, animals were only exposed for 100 minutes and should also be essentially free of TRI after 24 hours. Furthermore, rats exposed to 500 ppm for 6 hours/day for 4 days had only trace amounts of TRI in the brain and blood 17 hours after the end of the exposure period (Savolainen *et al.*, 1977). The observation that rapid elimination also occurs from the brain is significant, since the lipophilicity of TRI could potentially delay its mobilization from lipoidal tissues. Additional evidence also suggests that solvents do not exhibit delayed mobilization from the brain. Perchloroethylene and toluene, two solvents with greater fat:blood partition coefficients than TRI (Gargas *et al.*, 1989), have been shown to be eliminated from the brains of rats and mice at comparable or slightly faster rates than from the blood (Dallas *et al.*, 1994a; Benignus *et al.*, 1981; Bruckner and Peterson, 1981).

The ability of rate-decreasing TRI concentrations to augment the effects of subsequent exposures is also not likely to stem from the accumulation of metabolites, since metabolism plays only a minor role in TRI elimination. Rats have been reported to metabolize only 2-6% of the TRI absorbed during 6 hour inhalation exposures to 150 and 1500 ppm (Schumann *et al.*, 1982a, 1982b). Furthermore, repeated daily exposure of rats to 1500 ppm for approximately 16 months does not discernably alter the extent of metabolism, routes of excretion, or tissue concentration of TRI when compared to age-matched, singly exposed animals (Schumann *et al.*, 1982b). These findings indicate that the brain deposition and pharmacokinetic profile of TRI were comparable in the singly

and repeatedly exposed animals at equivalent exposure concentrations in the current study. That solvent-free responding was unaffected by repeated exposure to TRI in itself discounts the role of parent compound or metabolite accumulation in the residual effects of TRI on schedule-controlled responding. If TRI and it's metabolites are present at the time of solvent-free responding, they must be so at pharmacologically inactive concentrations.

That an animal's response to solvent exposure can be influenced by its behavioral and exposure history has been previously suggested by Liang *et al.* (1983) who determined cumulative concentration-effect functions in mice on five successive days for carbon disulphide's (CS_2) effect on the interruption of a light beam maintained by a FI-60 schedule of milk presentation. Two years earlier, Glowa (1981) had reported the results of a very similar experiment with toluene. The rate-decreasing effects of CS_2 and toluene became progressively greater with repeated daily exposure, even though parent compound and metabolite accumulation was discounted as a possible cause. Another possible explanation for the residual effects of CS_2 and toluene, as well as for TRI in the present study, is that an aversion to responding developed as a result of pairing possibly irritant or toxic concentrations of the solvents with operant stimuli. While this was a viable explanation for why the repeated administration of the pesticide chlordimeform augments its behavioral toxicity and severely decreases control responding (Glowa, 1986), it too can be largely discounted on the basis that CS_2, toluene and TRI exposure failed to affect solvent-free responding.

Although previous exposure to TRI clearly affects behavioral responsiveness to subsequent exposures, ample evidence exist that suggest that this is not a generalized phenomenon. For example, Glowa and Dews (1987) have shown that the cumumulative dose-response curve for ethyl acetate on schedule-controlled responding in mice previously exposed to acetone is nearly identical to that obtained in exposure-naive mice. Likewise, the effects of toluene on schedule-controlled responding in mice previously

exposed to acetone, ethyl acetate, and methyl ethyl ketone were similar to those in mice exposed initially to toluene (Glowa and Dews, 1987). Furthermore, the concentration-effect curve for cumene on schedule-controlled responding in experimentally-naive mice is superimposable on the curve generated with mice previously exposed to other alkyl benzenes (Tegeris, 1991). However, since both current and previous results (Glowa *et al.*, 1983; Tegeris, 1991) indicate that repeated and cumulative dosing may result in a shift of the concentration-effect curve to the left of that generated with single exposures, care should be taken when designing and interpreting studies evaluating the dose-dependency of solvent-induced behavioral effects.

The comparison of dose-response curves as a method of investigation in behavioral toxicology is labor intensive, but has proven to be extremely informative in the current and previous studies (Glowa and Dews, 1983; Glowa *et al*, 1983; Glowa, 1990; Tegeris, 1991). The results of the current investigation suggest that the residual effect observed may be due to the behavioral effect of TRI itself, that is, it may be more likely to occur following exposure to concentrations that decrease responding. These data support the well established belief that an animal's response to a drug or chemical can be modified by its prior behavioral and exposure history, even when such history is not reflected during exposure-free periods (Barrett, 1977; Glowa and Barrett, 1983).

REFERENCES

Arlien-Soborg, P. (1992). *Solvent Neurotoxicity*. CRC Press, Inc., Boca Raton, FL., 61-106.

(ATSDR) Agency for Toxic Substances and Disease Registry. (1994). *Toxicological Profile for 1,1,1-Trichloroethane*. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Balster, R.L. (1987). Abuse potential evaluation of inhalants. *Drug and Alcohol Dependence*. 49, 7-15.

Balster, R.L., Moser, V.C., and Woolverton, W.L. (1982). Concurrent measurements of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J. Pharmacol. Methods*. 8, 299-309.

Barrett, J.E. (1977). Behavioral history as a determinant of the effects of d-amphetamine on punished behavior. *Science*. 198, 67-69.

Benignus, V.A., Muller, K.E., Barton, C.N., and Bittikofer, J.A. (1981). Toluene levels in blood and brain of rats during and after respiratory exposure. *Toxicol. Appl. Pharmacol.* 61, 326-334.

Bruckner, J.V., and Peterson, R.G. (1981). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol. Appl. Pharmacol.* 61, 27-38.

Claudio, L. (1992). An analysis of the U.S. Environmental Protection Agency neurotoxicity testing guidelines. *Regul. Toxicol. Pharmacol.* 16, 202-212.

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. (1994a). Use of tissue disposition data from rats and dogs to determine species differences in input parameters for a physiological model for perchloroethylene. *Environ. Res.* 67, 54-67.

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. (1994b). Development of a physiologically based pharmacokinetic model for perchloroethylene using tissue concentration-time data. *Toxicol. Appl. Pharmacol.* 128, 50-59.

Evans, E.B., and Balster, R.L. (1991). CNS depressant effects of volatile organic solvents. *Neurosci. Biobehav. Rev.* 15, 233-241.

Gargas, M.L., Burgess, R.J., Voisard, D.E., Cason, G.H., and Andersen, M.E. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 98, 87-99.

Glowa, J.R. (1981). Some effects of sub-acute exposure to toluene on schedule-controlled behavior. *Neurobehav. Toxicol. Teratol.* 3, 463-465.

Glowa, J.R. (1985). Behavioral effects of volatile organic solvents. In: *Behavioral Pharmacology: The Current Status*. Alan R. Liss, Inc., 537-552.

Glowa, J.R. (1986). Acute and sub-acute effects of deltamethrin and chlordimeform on schedule-controlled responding in the mouse. *Neurobehav. Toxicol. Teratol.* 8, 97-102.

Glowa, J.R. (1990). Behavioral toxicology of solvents. *Drug Dev. Res.* 20, 411-428.

Glowa, J.R. (1991). Behavioral toxicology of volatile organic solvents V. Comparisons of the behavioral and neuroendocrine effects among n-Alkanes. *J. Am. Coll. Toxicol.* 10(6), 639-646.

Glowa, J.R. (1993). Behavioral and neuroendocrine effects of diethyl ether exposure in the mouse. *Neurotoxicol. Teratol.* 15, 215-221.

Glowa, J.R., and Barrett, J.E. (1983). Drug history modifies the behavioral effects of pentobarbital. *Science.* 220, 333-335.

Glowa, J.R., and Dews, P.B. (1983). Behavioral toxicology of organic solvents. II. Comparison of results on toluene by flow-through and closed chamber procedures. *J. Am. Coll. Toxicol.* 2(4), 319-323.

Glowa, J.R., and Dews, P.B. (1987). Behavioral toxicology of volatile organic solvents. IV. Comparisons of the rate-decreasing effects of acetone, ethyl acetate, methyl ethyl ketone, toluene, and carbon disulphide on schedule-controlled behavior of mice. *J. Am. Coll. Toxicol.* 6(4), 461-469.

Glowa, J.R., DeWeese, M.E., Natale, M.E., Holland, J.J., and Dews, P.B. (1983). Behavioral toxicology of volatile organic solvents. I. Methods: acute effects. *J. Am. Coll. Toxicol.* 2, 175-185.

Liang, Y-X, Glowa, J.R., and Dews, P.B. (1983). Behavioral toxicology of volatile organic solvents. III. Acute and subacute effects of carbon disulphide exposure on the behavior of mice. *J. Am. Coll. Toxicol.* 2(6), 379-389.

Mackay, C.J., Campbell, L., Samuel, A.M., Alderman, K.J., Idzikowski, C., Wilson, H.K., and Gompertz, D. (1987). Behavioral changes during exposure to 1,1,1-trichloroethane: Time-course and relationship to blood solvent levels. *Am. J. Ind. Med.* 11, 223-239.

Moser, V.C., and Balster, R.L. (1986). The effects of inhaled toluene, halothane, 1,1,1-trichloroethane, and ethanol on fixed-interval responding in mice. *Neurobehav. Toxicol. Teratol.* 8, 525-531.

Rice, D.C. (1988). Quantification of operant behavior. *Toxicol. Lett.* 43, 361-379.

Sanger, D.J., and Blackman, D.E. (1976). Theoretical review. Rate-dependent effects of drugs: A review of the literature. *Pharmacol. Biochem. Behav.* 4, 73-83.

Savolainen, H., Pfaffli, P., Tengen, M., and Vainio, H. (1977). Trichloroethylene and 1,1,1-trichloroethane: effects on brain and liver activity after five days intermittent inhalation. *Arch. Toxicol.* 38, 229-237.

Schumann, A.M., Fox, T.R., and Watanabe, P.G. (1982a). [^{14}C]Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. *Toxicol. Appl. Pharmacol.* 62, 390-401.

Schumann, A.M., Fox, T.R., and Watanabe, P.G. (1982b). A comparison of the fate of inhaled methyl chloroform (1,1,1-trichloroethane) following single or repeated exposure in rats and mice. *Fund. Appl. Toxicol.* 2, 27-32.

Sette, W.F., and Levine, T.F. (1986). Behavior as a regulatory endpoint. In: *Neurobehavioral Toxicology.* (Ed. Annau, Z.). The Johns Hopkins University Press, 391-403.

Sidman, M. (1960). *Tactics of Scientific Research.* Authors Cooperative, Inc., Boston, MA., 42-67.

Tegeris, J.S. (1991). Acute behavioral effects of alkylbenzenes evaluated utilizing a functional observational battery and schedule-controlled operant behavior. Doctoral Dissertation. Virginia Commonwealth University.

Wallenstein, S., Zucker, C.L., and Fleiss, J. (1980). Some statistical methods useful in circulation research. *Circ. Res.* 47(1), 1-9.

Warren, D.A., Dallas, C.E., Reigle, T.G., and Christmus, W.H. (1993). Behavioral changes during 1,1,1-trichloroethane (TRI) inhalation in rats: Relationship to brain and blood levels. *The Toxicologist.* 13, 248.

Weiss, B., and Cory-Slechta, D.A. (1994). Assessment of behavioral toxicity. In: *Principles and Methods of Toxicology, Third Edition.* (Ed. Hayes, A.W.). Raven Press, Ltd., New York, 1091-1155.

Wenger, G.R. (1990). Operant behavior as a technique for toxicity testing. *Neurotoxicol. Teratol.* 12, 515-521.

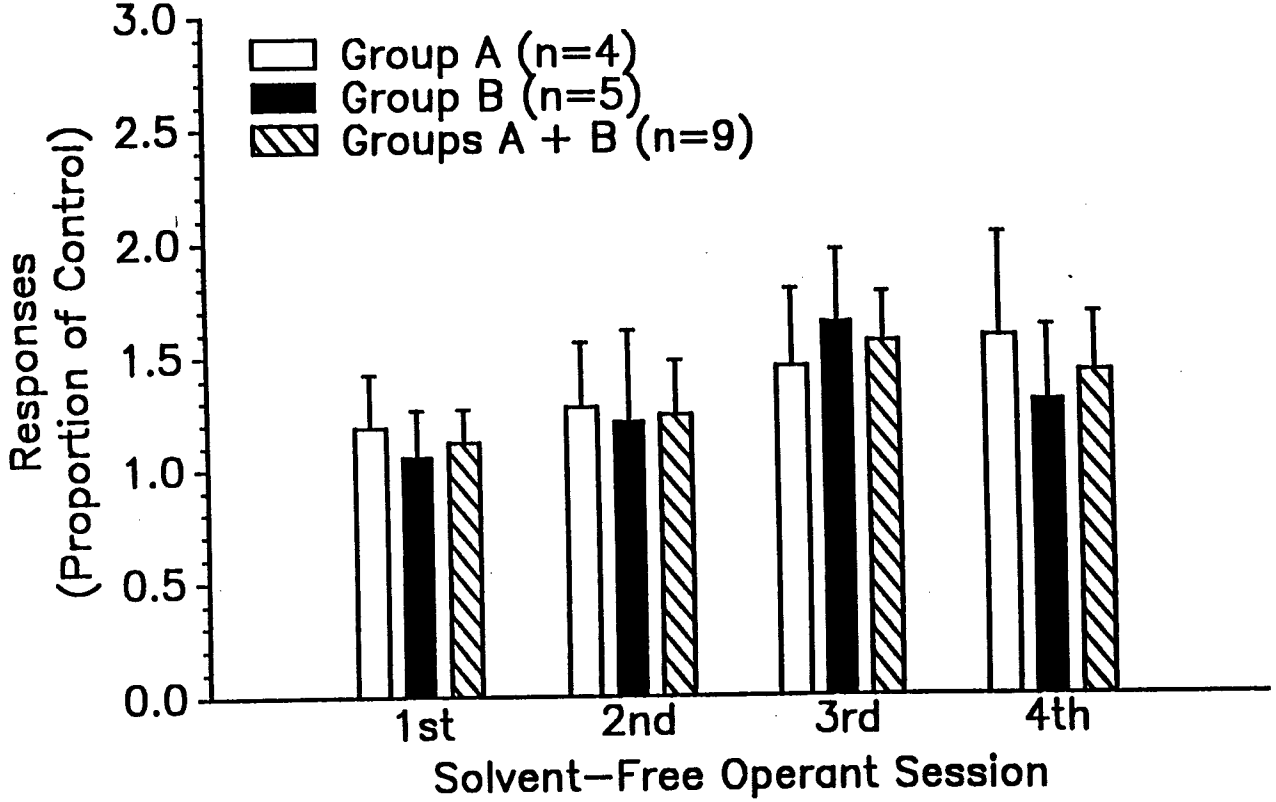

Figure 1: Number of responses during the last 100 minutes of solvent-free operant sessions that were conducted 24 hours after each exposure, reported as a proportion of control. Data are the mean ± SE of repeatedly exposed rats in Group A, Group B, and Groups A + B.

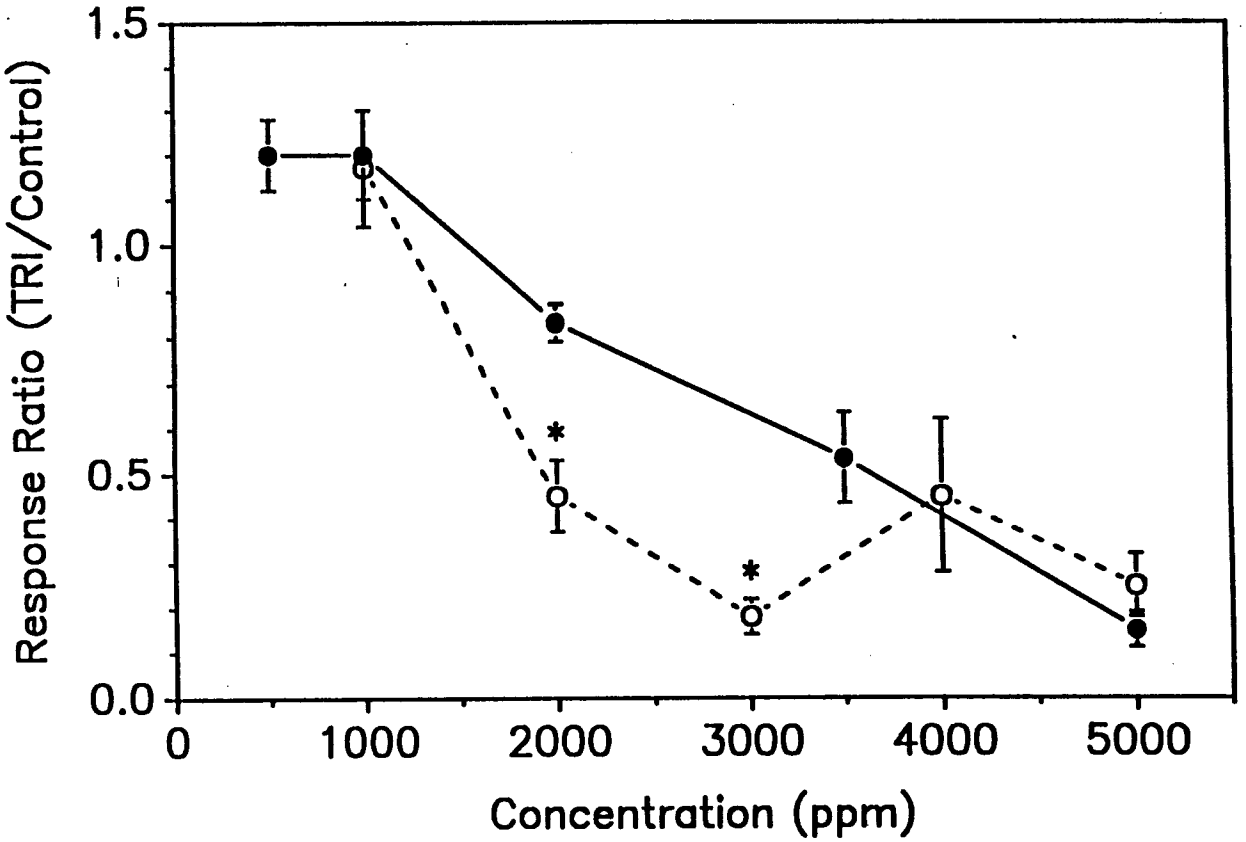

Figure 2: Dose-response curves for the effect of TRI on operant responding. Each closed circle (•) represents the mean operant response ratio ± SE for five chemically-naive rats. Each open circle (o) represents the mean operant response ratio ± SE for the nine rats repeatedly exposed to TRI. Asterisks signify concentrations at which the responses of singly and repeatedly exposed rats significantly differ ($p < 0.0005$).

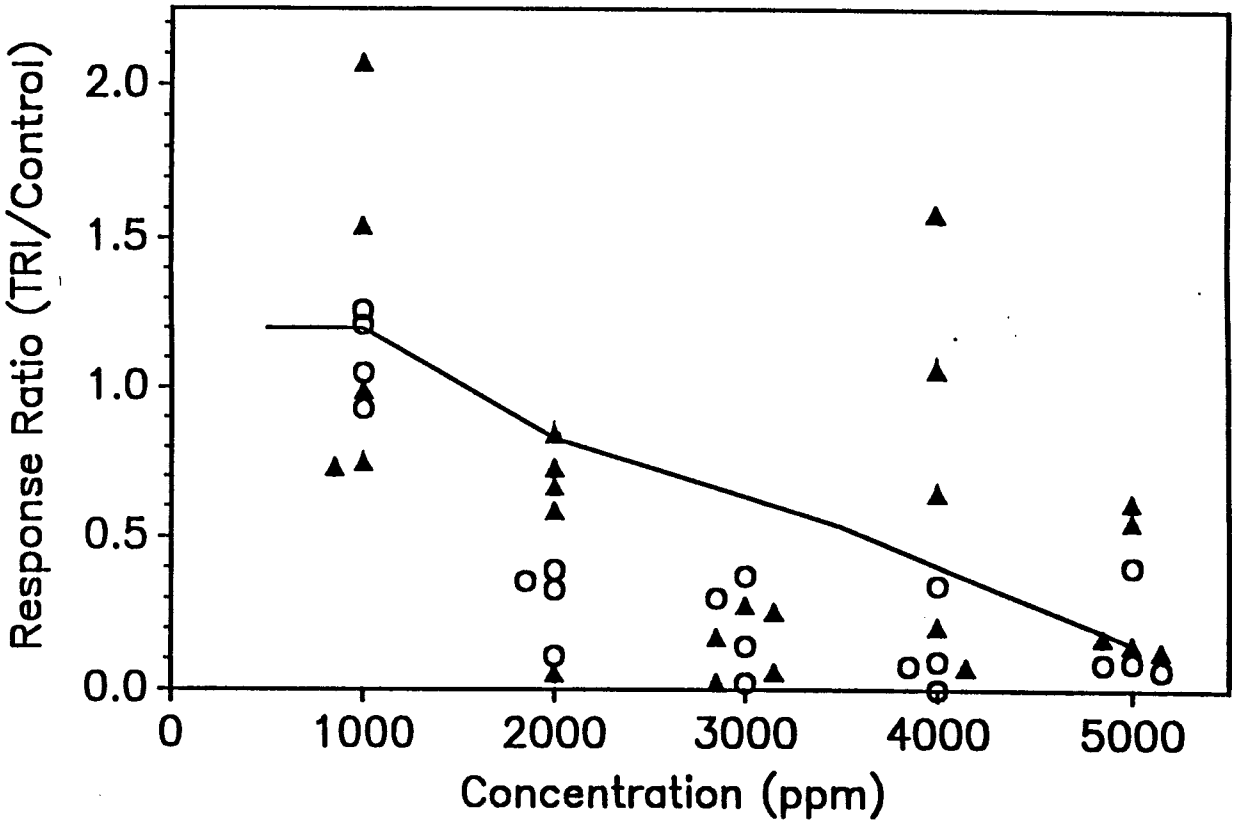

Figure 3: Operant response ratios of repeatedly exposed rats relative to a dose-response curve generated with singly exposed rats (solid line). Each open circle (o) represents one of four rats exposed in the order 1000, 5000, 3000, 4000 and 2000 ppm (Group A). Each closed triangle (▲) represents one of five rats exposed in the order 4000, 1000, 5000, 3000 and 2000 ppm (Group B). Symbols have been offset for clarity.

BIPHASIC EFFECTS OF 1,1,1-TRICHLOROETHANE ON THE LOCOMOTOR ACTIVITY OF MICE: RELATIONSHIP TO BLOOD AND BRAIN SOLVENT CONCENTRATIONS[1]

[1]Warren, D.A., Bowen, S.E., Dallas, C.E., and Balster, R.L. To be submitted to *Neurotoxicology and Teratology*.

ABSTRACT

Despite the central nervous system (CNS) being a target of virtually all solvents, there have been few studies of solvent effects on unlearned animal behaviors. Little is known about the relationship of exposure concentration to behavioral effect, and quantitative data relating the toxicologically important target organ (i.e., brain) dose to behavioral effect are almost non-existent. To examine the relationships of blood and brain concentrations of 1,1,1-trichloroethane (TRI) to locomotor activity, mice were exposed to TRI (500-14,000 ppm) in static inhalation chambers for 30 minutes, during which locomotor activity was measured. Separate mice were exposed to the same concentrations for 6, 12, 18, 24 and 30 minutes to determine blood and brain concentration versus time profiles for TRI. The lowest TRI concentration studied (500 ppm) had no effect on activity, intermediate concentrations (1000-8000 ppm) increased activity immediately to levels that remained constant over time, and higher concentrations (10,000-14,000 ppm) produced biphasic effects, i.e., increases in activity followed by decreases. TRI concentrations in blood and brain approached steady-state equilibria very rapidly, demonstrated linear kinetics, and increased in direct proportion to one another. Locomotor activity increased monophasically ($\approx$ 3.5 fold) as solvent concentrations increased from approximately 10-160 μg/g brain and μg/ml blood. As concentrations exceeded the upper limit of this range, the activity level declined and eventually fell below the control activity level at approximately 250 μg/g brain and μg/ml blood. The broad dose range employed demonstrated that TRI, like some classical CNS depressants, is capable of producing biphasic effects on behavior, supporting the hypothesis that selected solvents are members of the general class of CNS depressant drugs. By relating internal dose measures to locomotor activity, our understanding of the effects observed and their predictive value may be enhanced.

INTRODUCTION

The extent of solvent exposure by inhalation ranges from low concentrations produced by the proper use of commercial products to dangerously high concentrations encountered during recreational solvent abuse and accidents or misuse in industry (Moser and Balster, 1985). Following high-level exposure, a general depression of the central nervous system (CNS) may occur, with effects ranging from impaired cognition and motor skills to death. Acute solvent exposures to human volunteers have impaired performance in tests of manual dexterity, eye-hand coordination, perceptual speed and reaction time, and produced lightheadedness and imbalance (Torkelson *et al.*, 1958; Stewart *et al.*, 1961; Gamberale and Hultengren, 1973; Mackay *et al.*, 1987). Exposure limits have been established to protect workers from such effects (ACGIH, 1993), but the majority of these is based on a limited number of poorly documented responses to occupational exposures, often with little or no experimental confirmation from animal studies. In addition, few animal studies have evaluated the toxicologic properties of solvents under exposure conditions typical of solvent abuse (Bruckner and Peterson, 1981; Balster, 1987).

Several committees of the National Academy of Sciences have recommended examinations of learned and unlearned animal behaviors (as well as morphology) as the first steps in chemical hazard identification and evaluation (Buckholtz and Panem, 1986). One means of evaluating a chemical's effect on unlearned behavior is by measuring changes in locomotor activity. Activity measurements have several advantages, including ready quantification, automation, speed and limited cost. Nonetheless, few reports of the effects of solvents on locomotor activity are available (Wood and Colotla, 1990).

With the exception of the prototypical aromatic hydrocarbon, toluene, the effects of 1,1,1-trichloroethane (TRI) on animal behavior are better characterized than those of other industrial solvents. 1,1,1-Trichloroethane has produced rate changes in the food-

reinforced lever-pressing of mice (Balster *et al.*, 1982; Moser and Balster, 1986), altered performance on a match-to-sample discrimination task in baboons (Geller *et al.*, 1982), and impaired the ability of rats to avoid shock by lever-pressing (Mullin and Krivanek, 1982) and mice to remain atop an inverted screen (Moser and Balster, 1985). In addition, a single study has been published documenting the effects of TRI on motor activity (Kjellstrand *et al.*, 1985). Of the four inhalation concentrations examined in the study, only two produced activity changes in mice during exposure, making the study of limited value in defining the dose-response relationship.

As behavior is increasingly being considered a potential regulatory endpoint, the need for dose-response determinations will likely increase to provide a basis for the development of rational limits for human exposure. Because of interspecies differences in pharmacokinetics, the extrapolation of potential human risk from animal toxicity data without some knowledge of the corresponding target organ dose or a dose surrogate, may involve uncertainty. This uncertainty can be reduced by data that describe, for example, the blood and brain concentrations of a solvent and their relationships to corresponding levels of behavioral toxicity. Unfortunately, few studies have generated data of this type.

In the present investigation, blood and brain solvent concentrations and locomotor activity were measured in mice during exposure to nine concentrations of TRI vapor ranging from 500-14,000 ppm. This exposure regimen, extreme in terms of the number and range of concentrations, enabled the relationship between locomotor activity and inhaled solvent concentration to be well characterized. More importantly, it enabled the relationships between locomotor activity and blood and brain solvent concentrations to be described for the first time. By relating internal dose measures to locomotor activity, our understanding of the effects observed and their predictive value may be enhanced. In addition, the use of a broad dose range demonstrated that TRI, like many classical CNS depressants, produces biphasic effects on some behaviors, supporting the hypothesis

that selected solvents are members of the general class of CNS depressant drugs (Evans and Balster, 1991).

MATERIALS AND METHODS

Chemicals: 1,1,1-Trichloroethane of 97%+ purity was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Fisher Scientific (Fair Lawn, NJ). Burdick and Jackson Brand, High Purity Solvent isooctane was obtained from Baxter Healthcare Corp. (Muskegon, MI).

Animals: Male, albino Swiss-Webster (CFW®) mice (25-35 g) were obtained from Charles River Breeding Laboratories in Raleigh, NC and Wilmington, MA. Mice were housed singly in polypropylene cages with hardwood chip bedding (Sani-Chips, Montville, NJ) and stainless steel wire lids in temperature- (23°C) and humidity- (45%) controlled rooms with 12-hour light-dark cycles (light: 0700-1900 hr). Mice were acclimated for at least 7 days prior to use, during which time food (Agway Prolab RMH 3000, Syracuse, NY) and tap water were available *ad libitum*. Following acclimation, ten mice on which locomotor activity measures were made were food deprived and maintained at 80-85% of their free-feeding weight to emulate mice whose operant behavior was examined under identical exposure conditions in a separate study. The mice on which locomotor activity measures were made had previously been exposed to toluene (250-8,000 ppm). Mice used for blood and brain concentration determinations were not food restricted and were chemically naive.

Exposure Apparatus: Inhalation exposures were conducted in several identical static chambers which have been described previously (Moser and Balster, 1986). The chambers were 29-liter chromatography jars (Pyrex® 6942) with acrylic covers. A fan projected into each chamber above a wire basket which supported a piece of filter paper. A mouse was placed in the bottom of the chamber, the top put in place, and a calculated

amount of solvent injected onto the filter paper which volatilized with the aid of the fan to produce the desired vapor concentration. Vapor concentrations reached target levels in 1-3 minutes and remained stable for the duration of exposure. Concentrations within the chambers were continuously monitored with a Miran 1A infrared spectrometer (The Foxboro Co., East Bridgewater, MA) during locomotor activity measurements, and by gas chromatographic analyses of air samples serially extracted with a 1.0 ml gas-tight syringe and a 12" needle during blood and brain concentration determinations. The extracted air samples were directly injected into a Shimadzu GC-14A gas chromatograph (GC) equipped with an electron capture detector (ECD) and TRI concentrations were calculated from standard curves based on atmospheres of known concentration prepared in 9-liter glass jars. Chromatographic analyses were conducted using a stainless-steel column (182 x 0.317 cm) packed with 3% OV-17 (100-120 mesh) (Alltech Associates, Inc., Deerfield, IL). The GC operating conditions were injection port temperature, 150°C; column temperature, 80°C; ECD temperature, 360°C; flow rate for argon:methane (95:5) carrier gas, 60 ml/minute.

Locomotor Activity Measurements: Ten mice were adapted to the static exposure chambers for 30 minutes/day for 5 days, after which locomotor activity was measured for 30 minutes beginning immediately upon vapor generation. Mice were free to move about inside the chambers and locomotor activity was measured via two sets of photocells that bisected each chromatography jar. During the 30-minute exposure periods, locomotor activity was defined as the total number of photocell breaks or counts. Locomotor activity was recorded in 1-minute bins in order to maximize the sensitivity to detect temporal changes. Although rearing in some cases may have been detected as activity, the majority of activity counts were due to horizontal movement. Test sessions were conducted twice a week (Tuesdays and Fridays), with continued placement in the exposure chambers with air only on the remaining weekdays. Data from all Thursdays were averaged and served as the control performance. Mice were exposed to TRI

concentrations (500, 1000, 2000, 4000, 6000, 8000, 10,000, 12,000 and 14,000 ppm) in an ascending order and to only one concentration per test day. All experiments were conducted during the light phase of the light-dark cycle and at the same time each day for each animal.

Blood and Brain Sampling: Blood and brain solvent concentrations were determined in mice during exposure to the same concentrations at which locomotor activity was measured. Mice received one 30-minute chamber adaptation period on the day prior to solvent exposure. At 6, 12, 18, 24 and 30 minutes after the start of exposure, a mouse was removed from the exposure chamber and sacrificed by CO_2 asphyxiation. Blood (0.2-0.5 ml) was withdrawn from the inferior vena cava with a 1 ml tuberculin syringe and a 25 gauge needle and whole brains collected ($\approx$ 0.4 g) within 1-2 minutes of sacrifice. Blood and brain samples were immediately placed into chilled scintillation vials containing 8 ml isooctane and 2 ml 0.9% saline. Four mice were sacrificed at each time point during exposure to each TRI concentration, except at 10,000 and 14,000 ppm only two mice were sacrificed at each time point.

Blood and Brain Analysis: Blood and brain samples were analyzed for TRI content by a method originally described by Chen *et al.* (1993). Briefly, blood and brain samples were homogenized as quickly as possible (5-10 seconds) with an Ultra-Turrax® homogenizer (Tekmar Co., Cincinnati, OH) to minimize volatilization of TRI, after which the samples were vortex-mixed for 30 seconds. The homogenates were then centrifuged at 2500 x g for 10 minutes at 4°C in the capped scintillation vials. An aliquot of the isooctane layer (5-10 μl) was either transferred directly to an 8 ml headspace vial or first diluted with isooctane. The vials were capped immediately with Teflon®-lined latex rubber septa in aluminum seals and crimped tightly. Analyses of TRI were made with a Sigma Model 300 GC (Perkin-Elmer Co., Norwalk, CT) equipped with a HS6 headspace sampler (headspace sampler temperature, 70°C) and an ECD under the same chromatographic conditions previously described. 1,1,1-Trichloroethane

concentrations were calculated from daily prepared standard curves made by diluting various amounts of TRI in isooctane and corrected for the percent recovery characteristic of blood and brain samples. The percent recovery of TRI from blood (95.5%) and brain (94.3%) samples was previously determined by You *et al.* (1994) by injecting freshly harvested blood and brain samples with 4 μl of a solution containing 1 mg TRI per 1 ml isooctane and analyzing the samples as previously described. The limit of detection for TRI was $\approx$ 0.5 ng in 8 ml of air.

Data Analysis: Locomotor activity (mean $\pm$ standard error (SE) of ten mice) during exposure to each TRI concentration was plotted relative to control activity. One-way analysis of variance was used to compare control activity to activity during TRI exposures of questionable effect (i.e., 500, 1000 and 2000 ppm). Areas under the blood and brain concentration versus time curves (AUC) were calculated by the trapezoidal rule (Rowland and Tozer, 1980), and maximum blood and brain concentrations (C_{max}) were obtained by visual inspection of data. Pooling data from the nine exposure concentrations, mean blood and brain solvent concentrations at 6, 12, 18, 24 and 30 minutes were plotted against each other, and the resulting scatter plot was subjected to least squares linear regression analysis and the degree of correlation measured by comparing the correlation coefficient to values in a t-distribution table (Gad and Weil, 1986). Pooling data once again from the nine exposure concentrations, mean blood and brain solvent concentrations at 6, 12, 18, 24 and 30 minutes were also plotted against locomotor activity (number of counts during exposure minus the number of counts during control) at corresponding times and exposure concentrations. The minimum level of significance was set at $p \leq 0.05$ for all statistical tests.

RESULTS

Locomotor activity during exposure to each of the nine TRI concentrations is shown relative to control activity in Figure 1. The results clearly demonstrate that inhalation of TRI produces concentration dependent biphasic stimulant and depressant effects on the locomotor activity of mice. Locomotor activity during exposure to 500 ppm did not differ from control activity ($F(1,58) = 0.497, p = 0.484$), which remained at a constant level for 30 minutes. Exposure to 1000 ppm did increase activity ($F(1,58) = 16.3, p = 0.002$), as did all concentrations in excess of this lowest-observed-effect-level. Exposures from 1000-8000 ppm elevated activity in the first minute of exposure and this increased activity was sustained at roughly the same level for the remainder of the exposure. Higher concentrations (10,000, 12,000 and 14,000 ppm) had a biphasic effect on locomotor activity. At these concentrations, activity initially increased monophasically with the time to peak activity level being dependent upon the inhaled concentration. With continued exposure, activity decreased at a rate that was also concentration dependent, and fell below the control level during the latter part of the exposure to 14,000 ppm. Only at 12,000 and 14,000 ppm did any mice cease activity continuously for greater than 1 minute. Ataxia was occasionally observed at the two highest concentrations. No seizures or fatalities occurred in any of the exposed animals.

The concentrations of TRI in blood and brain as a function of the degree and duration of exposure are shown in Table 1, and the C_{max} and AUC values based on these data are shown in Table 2. As expected from the abrupt changes in locomotor activity upon the initiation of exposure, TRI was rapidly absorbed from the lung as evidenced by its substantial presence in blood and brain after just 6 minutes of exposure. 1,1,1-Trichloroethane concentrations in blood and brain approached steady-state equilibria very rapidly, as blood and brain concentrations at 6 minutes averaged 77% of those at 30 minutes, and those at 12 minutes rarely differed from those at 30 minutes. With a few

isolated exceptions, TRI demonstrated linear kinetics over the broad dose range as blood and brain concentrations and AUC and C_{max} values were roughly proportional to the inhaled TRI concentrations.

As expected for a well-perfused and lipid-rich organ such as the brain, its pattern of TRI accumulation was very similar to that of the blood. The scatter plot relating mean blood and brain solvent concentrations (Figure 2) clearly illustrates that brain levels of TRI increase in direct proportion to blood levels ($\approx$ 1:1), making blood and brain solvent concentrations highly correlated ($r = 0.97$, $df = 43$, $t = 24.89$, $p < 0.001$). These findings strongly suggest that blood and brain TRI concentrations are equally suited to relate to locomotor activity.

The scatter plots relating mean blood and brain solvent concentrations to locomotor activity (Figures 3 and 4) illustrate a monophasic increase in activity as solvent concentrations increase to approximately 160 μg/g brain and μg/ml blood. At higher solvent concentrations, activity decreases before eventually falling below the control level at approximately 250 μg/g brain and μg/ml blood. Based on the no-observed-effect-level of 500 ppm and the lowest-observed-effect-level of 1000 ppm, the estimated threshold concentration for locomotor activity effects is 10-20 μg/g brain and μg/ml blood.

DISCUSSION

Consistent with that shown for inhaled toluene and ip-administered ethanol (Hinman, 1987; Wood and Colotla,1990; Middaugh *et al.*, 1992), the results of this study indicate that inhaled TRI produces concentration dependent biphasic effects on locomotor activity. The lowest concentration studied (500 ppm) had no effect on activity, intermediate concentrations (1000-8000 ppm) increased activity immediately to levels that remained constant over time, and higher concentrations (10,000-14,000 ppm) produced biphasic effects, i.e., increases in activity followed by decreases. In the only

other published study of TRI's effect on motor activity, groups of five mice were repeatedly exposed to either TRI (890, 1300, 2000 and 4000 ppm), methylene chloride, perchloroethylene, toluene or trichloroethylene for 1 hour (Kjellstrand *et al.*, 1985). The authors described the activity pattern for TRI as "...simple, consisting of an increase and a decrease closely related to the increase and decrease of the chamber concentration." They also noted that TRI was less effective at increasing activity than the other solvents, as it was effective only at the two highest concentrations tested. In an unpublished study, Albee *et al.* (1990) also observed an increase in the motor activity of rats exposed to 4000 ppm TRI for 6 hours per day for 4 days (ATSDR, 1994). Additional evidence of a biphasic effect for TRI comes from two studies of schedule-controlled operant behavior in which both increases and decreases in the rate of lever-pressing for milk delivery were observed during and following inhalation exposure (Moser and Balster, 1986; Warren *et al.*, 1993).

Biphasic effects have also been documented in humans for TRI, as well as for other volatile, lipophilic agents such as ethyl alcohol, halothane and xylene (Kalant, 1978; Roth, 1979; Seppalainen *et al.*, 1981). Body sway was decreased and reaction time improved in volunteers exposed to 200 ppm TRI, while both deteriorated at 400 ppm (Arlien-Soborg, 1992). 1,1,1-Trichloroethane's biphasic effect on human equilibrium has also been reported elsewhere (Savolainen *et al.*, 1982). In addition, exposure to 175 and 350 ppm TRI actually enhanced performance of volunteers in a test of distractability of attention and concentration (Mackay *et al.*, 1987).

Since most biphasic effects in humans have been on the vestibular system or neurophysiological parameters such as electroencephalograms and evoked potentials, there is little doubt that they are related to the accumulation of agents in the CNS. Many behavioral effects in laboratory animals, however, while generally considered to indicate specific neurological effects, could be produced by actions on other organ systems or the sensory-stimulative properties of the agents themselves (Tilson and Mitchell, 1984).

Recognizing this, Kjellstrand *et al.* (1985) exposed mice to an intense odorant stimulus (cologne) that was without effect on the locomotor activity of mice, indicating that solvent effects on activity were unlikely due to odor. This is further supported by observations of increased activity when toluene and ethanol are administered by a route other than inhalation (Wood and Colotla, 1990; Middaugh *et al.*, 1992). Since the lowest-observed-adverse-effect-level for acute TRI exposure in any rodent organ system other than the CNS is 8000 ppm (ATSDR, 1994), it is also unlikely that effects on other organ systems are responsible for TRI-induced activity changes. Additionally, solvent-induced toxicities other than the classical behavioral syndrome are usually metabolite mediated, and TRI is metabolized to a very limited degree (Schumann *et al.*, 1982a; 1982b). Moreover, activity increases, as opposed to decreases, are difficult to interpret as the mere disruption of activity by a nonspecific stressor such as irritation. Rather, activity increases would appear to reflect a biologically relevant event in the CNS, such as activation of the hypothalamo-pituitary-adrenal axis or the release of higher center control (Glowa, 1990). Therefore, we believe the biphasic effect observed in the present study was dependent on the deposition of TRI in the brain.

Hinman (1987) reported that toluene's biphasic effect on locomotor activity was consistent with the hypothesis that such behavior is dependent on the level of toluene in the CNS. He concluded that locomotor activity is increased at low CNS levels, while at higher CNS levels the hyperactivity is attenuated. Since Hinman's conclusion was based on a comparison of his locomotor activity data to time-courses of blood and brain concentrations determined by other investigators (Bruckner and Peterson, 1981; Benignus *et al.*, 1981), no attempt was made to quantitatively relate internal solvent concentrations to locomotor activity. On the basis of the present study, however, we can conclude that locomotor activity counts increase roughly 3.5 fold as TRI concentrations increase from approximately 10-160 μg/g brain and μg/ml blood. As concentrations exceed the upper limit of this range, activity levels decline until they reach control level at approximately

250 μg/g brain and μg/ml blood. As expected for a well perfused organ with a tissue:blood partition coefficient near unity (0.80) (Reitz *et al.*, 1987), brain TRI concentrations seemingly paralleled those of the blood. As a result, blood TRI concentrations also appear to be a reliable index of the locomotor activity level, agreeing with similar conclusions made in correlative studies of other behaviors with toluene and TRI (Bruckner and Peterson, 1981; Warren *et al.*, 1993).

Regardless of the behavior being examined, few studies have attempted to relate solvent pharmacokinetics to pharmacodynamics. In two such studies, blood and brain toluene concentrations in mice were highly correlated with performance in tests of reflexes and unconditioned behaviors (Bruckner and Peterson, 1981), as were blood and brain TRI concentrations in rats to the rate of schedule-controlled operant responding for milk delivery (Warren *et al.*, 1993). Also, Kishi *et al.* (1993) have reported a relationship between blood 1,1,2-trichloroethylene levels and shock avoidance performance decrements in rats. In studies of controlled human exposures, blood levels of m-xylene and TRI were measured and related to impaired body balance, eye tracking deficits and altered reaction times (Riihimaki and Savolainen, 1980; Mackay *et al.*, 1987). The present study is thought to be the first to relate internal dose measures of an industrial solvent to locomotor activity.

Given the diversity of neural influences on locomotor activity and the ubiquitous distribution of solvents within the brain (Rafales, 1986; Gospe and Calaban, 1988; Ameno *et al.*, 1992), studies of solvent effects on locomotor activity usually do not allow for a determination of mechanisms within the CNS. The value of locomotor activity may instead lie in its economy and sensitivity to solvent-induced changes. For example, the lowest behaviorally-active toluene concentration to date in animals increases locomotor activity (Wood and Colotla, 1990), and the lowest-observed-effect-level in the present study is about one-half that necessary to produce changes in schedule-controlled operant behavior under some reinforcement schedules (Balster *et al.*, 1982; Moser and Balster,

1986). Therefore, as was demonstrated in the present study, locomotor activity may be useful for the routine determination of target tissue dose-response relationships that will enable behavioral modifications observed in animals to be extrapolated to humans with a greater degree of certainty.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists) (1993). *Documentation of the Threshold Limit Values and Biological Exposure Indices.* Cincinnati, OH.

Albee, R.R., Mattsson, J.L., Beekman, M.J., et al. (1990). Acute motor activity of 1,1,1-trichloroethane in rats. Final Report. The Dow Chemical Company, Midland, MI. Fiche # OTS533134 [Unpublished Study].

Ameno, K., Kiriu, T., Fuke, C., Ameno, S., Shinohara, T., and Ijiri, I. (1992). Regional brain distribution of toluene in rats and in human autopsy. *Arch. Toxicol.* 66, 153-156.

Arlien-Soborg, P. (1992). *Solvent Neurotoxicity.* CRC Press, Inc., Boca Raton, FL., 291-306.

ATSDR (Agency for Toxic Substances and Disease Registry). (1994). *Toxicological Profile for 1,1,1-Trichloroethane.* U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Balster, R.L. (1987). Abuse potential evaluation of inhalants. *Drug and Alcohol Dependence.* 49, 7-15.

Balster, R.L., Moser, V.C., and Woolverton, W.L. (1982). Concurrent measurements of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *J. Pharmacol. Methods.* 8, 299-309.

Benignus, V.A., Muller, K.E., Barton, C.N., and Bittikofer, J.A. (1981). Toluene levels in blood and brain of rats during and after respiratory exposure. *Toxicol. Appl. Pharmacol.* 61, 326-334.

Bruckner, J.V., and Peterson, R.G. (1981). Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol. Appl. Pharmacol.* 61, 27-38.

Buckholtz, N.S., and Panem, S. (1986). Regulation and evolving science: Neurobehavioral toxicology. *Neurobeh. Toxicol. Teratol.* 8, 89-96.

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. (1993). Analyses of volatile C_2 haloethanes and haloethenes in tissues: sample preparation and extraction. *J. Chromatogr.* 612, 199-208.

Evans, E.B., and Balster, R.L. (1991). CNS depressant effects of volatile organic solvents. *Neurosci. Biobehav. Rev.* 15, 233-241.

Gad, S., and Weil, C.S. (1986). *Statistics and Experimental Design for Toxicologists.* Telford Press, Caldwell, New Jersey.

Gamberale, F., and Hultengren, M. (1973). Methylchloroform exposure II. Psychophysiological functions. *Scand. J. Work Environ. Health.* 10, 82-92.

Geller, I., Mendez, V., Hartmann, R.J., Gause, E., and Rippstein, W.J. (1982). Effects of 1,1,1-trichloroethane on a match-to-sample discrimination task in the baboon. *J. Tox. Env. Health.* 9, 783-795.

Glowa, J.R. (1990). Behavioral toxicology of solvents. *Drug Dev. Res.* 20, 411-428.

Gospe, S.M., and Calaban, M.J. (1988). Central nervous system distribution of inhaled toluene. *Fund. Appl. Toxicol.* 11, 540-545.

Hinman, D.J. (1987). Biphasic dose-response relationship for effects of toluene inhalation on locomotor activity. *Pharmacol. Biochem. Behav.* 26, 65-69.

Kalant, H. (1978). Alcohol and electrophysiology of the central nervous system. In: *Advances In Pharmacology And Therapeutics.* 8, 199-209. *Drug-Action Modification Comparative Pharmacology.* (Ed. Olive, G). Pergamon Press, Oxford.

Kishi, R., Harabuchi, I., Ikeda, T., Katakura, Y., and Miyake, H. (1993). Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans. *Br. J. Ind. Med.* 50, 470-480.

Kjellstrand, P., Holmquist, B., Jonsson, I., Romare, S., and Mansson, L. (1985). Effects of organic solvents on motor activity in mice. *Toxicology.* 35, 35-46.

Mackay, C.J., Campbell, L., Samuel, A.M., Alderman, K.J., Idzikowski, C., Wilson, H.K., and Gompertz, D. (1987). Behavioral changes during exposure to 1,1,1-trichloroethane: Time-course and relationship to blood solvent levels. *Am. J. Ind. Med.* 11, 223-239.

Middaugh, L.D., Bao, K., and Shepherd, C.L. (1992). Comparative effects of ethanol on motor activity and operant behavior. *Pharmacol. Biochem. Behav.* 43, 625-629.

Moser, V.C., and Balster, R.L. (1985). Acute motor and lethal effects of inhaled toluene, 1,1,1-trichloroethane, halothane and ethanol in mice: Effects of exposure duration. *Toxicol. Appl. Pharmacol.* 77, 285-291.

Moser, V.C., and Balster, R.L. (1986). The effects of inhaled toluene, halothane, 1,1,1-trichloroethane, and ethanol on fixed-interval responding in mice. *Neurobehav. Toxicol. Teratol.* 8, 525-531.

Mullin, L.S., and Krivanek, N.D. (1982). Comparison of unconditioned reflex and conditioned avoidance tests in rats exposed by inhalation to carbon monoxide, 1,1,1-trichloroethane, toluene or ethanol. *NeuroTox.* 3, 126-137.

Rafales, L.S. (1986). Assessment of Locomotor Activity. In: *Neurobehavioral Toxicology.* (Ed. Annau, Z.). The Johns Hopkins University Press, 54-68.

Reitz, R.H., Nolan, R.J., and Schumann, A.M. (1987). "Development of multispecies, multiroute pharmacokinetic models for methylene chloride and 1,1,1-trichloroethane (methyl chloroform)." In: *Drinking Water and Health.* 8, 8-26. National Academy Press, Washington, DC.

Riihimaki, V., and Savolainen, K. (1980). Human exposure to m-xylene. Kinetics and acute effects on the central nervous system. *Ann. Occup. Hyg.* 23, 411-422.

Rowland, M., and Tozer, T.N. (1980). Appendix B. Assessment of Area. In: *Clinical Pharmacokinetics: Concepts and Applications.* Lea and Febiger, Philadelphia, pp. 288-291.

Roth, H.R. (1979). Physical mechanisms of anesthesia. *Ann. Rev. Pharmacol. Toxicol.* 19, 159-178.

Savolainen, K., Riihimaki, V., and Laine, A. (1982). Biphasic effects of inhaled solvents on human equilibrium. *Acta. Pharmacol. et Toxicol.* 51, 237-242.

Schumann, A.M., Fox, T.R., and Watanabe, P.G. (1982a). [^{14}C]Methyl chloroform (1,1,1-trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. *Toxicol. Appl. Pharmacol.* 62, 390-401.

Schumann, A.M., Fox, T.R., and Watanabe, P.G. (1982b). A comparison of the fate of inhaled methyl chloroform (1,1,1-trichloroethane) following single or repeated exposure in rats and mice. *Fund. Appl. Toxicol.* 2, 27-32.

Seppalainen, A.M., Savolainen, K., and Kovala, T. (1981). Changes induced by xylene and alcohol in human evoked potentials. *Electroenceph. Clin. Neurophysiol.* 48, 64-72.

Stewart, R.D., Gay, H.H., Erley, D.S., Hake, C.L., and Schaffer, A.W. (1961). Human exposure to 1,1,1-trichloroethane vapour: relationship of expired air and blood concentrations. *Am. Ind. Hyg. Assoc. J.* 22, 252-262.

Tilson, H.A., and Mitchell, C.L. (1984). Neurobehavioral techniques to assess the effects of chemicals on the nervous system. *Ann. Rev. Pharmacol. Toxicol.* 24, 425-450.

Torkelson, T.R., Oyen, F., McCollister, D.D., and Rowe, V.K. (1958). Toxicity of 1,1,1-trichloroethane as determined on laboratory animals and human subjects. *Am. Ind. Hyg. Assoc. J.* 19, 353-362.

Warren, D.A., Dallas, C.E., Reigle, T.G., and Christmus, W.H. (1993). Behavioral changes during 1,1,1-trichloroethane (TRI) inhalation in rats: Relationship to brain and blood levels. *The Toxicologist.* 13, 248.

Wood, R.W., and Colotla, V.A. (1990). Biphasic changes in mouse motor activity during exposure to toluene. *Fund. Appl. Toxicol.* 14, 6-14.

You, L., Dallas, C.E., and Muralidhara, S. (1994). The pharmacokinetics of inhaled 1,1,1-trichloroethane following high milk intake in mice. *Drug Chem. Toxicol.* 17(4), 479-498.

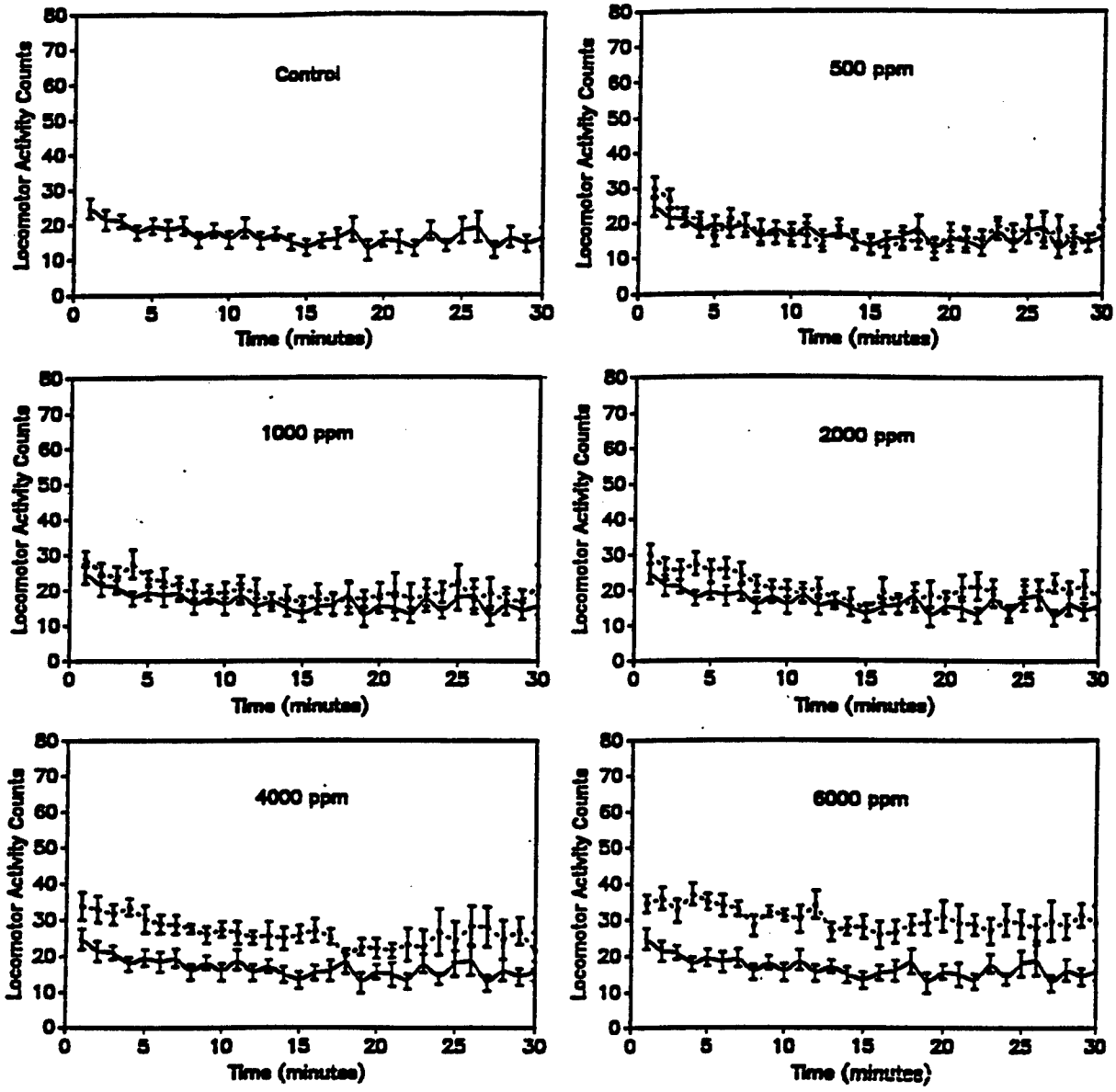

Figure 1: Locomotor activity during exposure to each of nine TRI concentrations (dashed lines) relative to control activity (solid lines). Each data point represents the mean $\pm$ SE of ten mice.

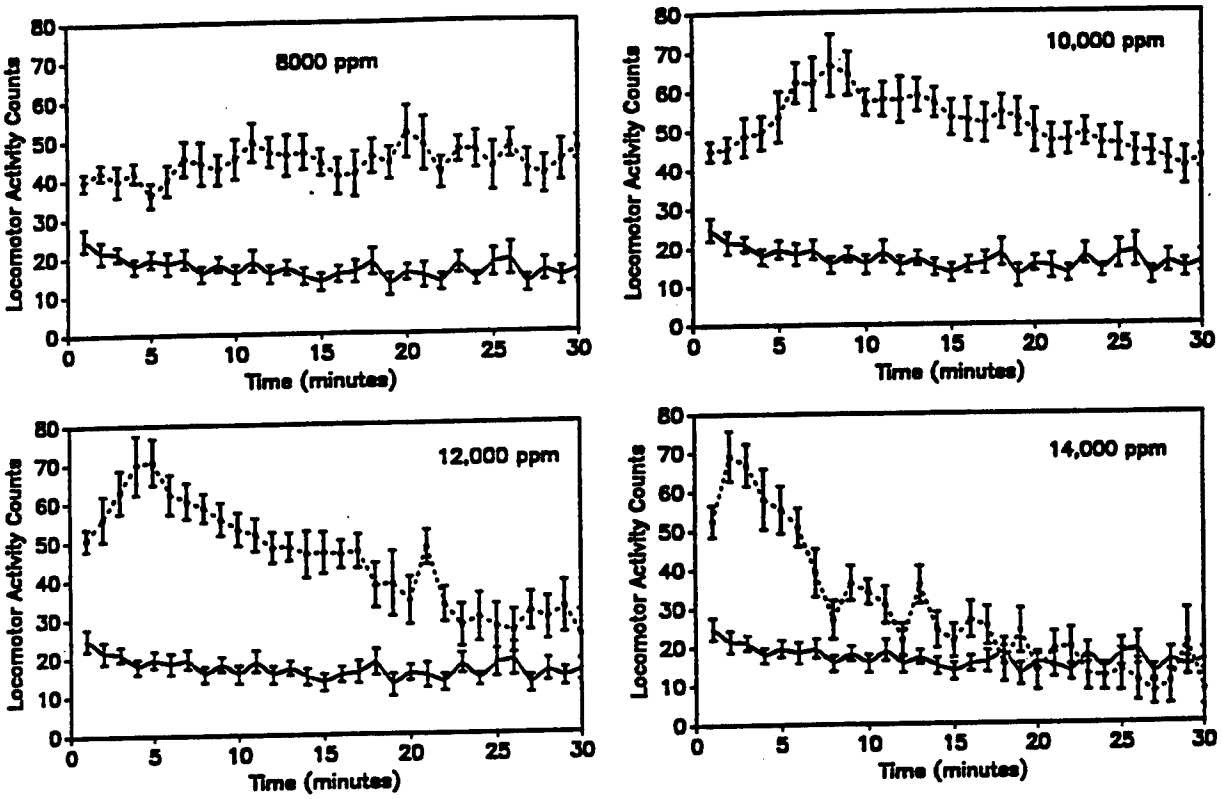

Figure 1 (Continued): Locomotor activity during exposure to each of nine TRI concentrations (dashed lines) relative to control activity (solid lines). Each data point represents the mean ± SE of ten mice.

Table 1. BLOOD AND BRAIN CONCENTRATIONS OF TRI DURING INHALATION EXPOSURE

Exposure Concentration (ppm)	Time (minutes)				
	6	12	18	24	30
500	8.8 ± 2.3 6.6 ± 0.4	9.8 ± 0.8 9.0 ± 0.4	10.7 ± 0.8 9.6 ± 0.1	10.9 ± 0.8 9.5 ± 0.8	10.2 ± 0.9 8.6 ± 0.7
1,000	17.5 ± 0.8 14.6 ± 0.7	17.5 ± 1.3 13.4 ± 2.0	20.4 ± 1.4 18.7 ± 0.7	22.6 ± 0.9 18.1 ± 0.8	19.6 ± 1.8 16.2 ± 0.9
2,000	25.8 ± 1.6 25.3 ± 0.8	32.2 ± 2.9 35.1 ± 3.6	35.4 ± 2.0 38.0 ± 3.9	34.7 ± 1.2 40.3 ± 2.6	37.2 ± 3.3 32.8 ± 0.9
4,000	52.6 ± 2.7 49.4 ± 2.3	49.7 ± 8.4 56.6 ± 9.3	52.3 ± 9.1 63.4 ± 4.1	52.3 ± 5.4 60.9 ± 4.5	67.2 ± 2.2 69.3 ± 5.4
6,000	60.5 ± 4.3 61.8 ± 3.4	88.3 ± 2.9 85.8 ± 4.1	97.6 ± 5.8 88.8 ± 2.0	81.1 ± 8.6 94.2 ± 5.3	96.1 ± 5.8 87.0 ± 4.9
8,000	109.7 ± 2.6 146.8 ± 11.1	124.5 ± 9.8 144.7 ± 6.0	120.8 ± 7.1 150.2 ± 4.7	122.1 ± 6.1 146.8 ± 20.3	138.1 ± 3.7 153.4 ± 4.4
10,000	137.8 ± 47.4 121.9 ± 3.1	161.8 ± 6.7 185.3 ± 6.1	165.3 ± 14.7 168.5 ± 12.1	195.8 ± 20.9 196.1 ± 30.7	204.6 ± 10.9 175.8 ± 16.1
12,000	156.0± 0.5 195.5 ± 21.7	237.0 ± 23.0 185.0 ± 6.2	224.5 ± 62.0 211.2 ± 11.7	214.0 ± 10.0 217.7 ± 6.8	224.5 ± 20.7 240.8 ± 10.9
14,000	195.6 ± 11.5 146.1 ± 9.6	239.3 ± 4.6 202.3 ± 15.0	250.8 ± 13.8 194.3 ± 1.1	256.5 ± 14.9 304.7 ± 41.4	257.7 ± 6.9 259.1 ± 26.1

Values are the mean ± SE of four mice, except at 10,000 and 14,000 ppm values are the mean ± SE of two mice. Brain concentrations of TRI are presented beneath blood concentrations.

Table 2. PHARMACOKINETIC PARAMETERS DURING INHALATION EXPOSURE

Exposure Concentration (ppm)	AUC (μg· min/ml) (blood)	AUC (μg· min/g) (brain)	C_{max} (μg/ml) (blood)	C_{max} (μg/g) (brain)
500	271.9	234.7	10.9 ± 0.8	9.6 ± 0.1
1,000	526.8	437.5	22.6 ± 0.9	18.7 ± 0.7
2,000	879.7	930.6	37.2 ± 3.3	40.3 ± 2.6
4,000	1441.9	1489.5	67.2 ± 2.2	69.3 ± 5.4
6,000	2253.5	2272.1	97.6 ± 5.8	94.2 ± 5.3
8,000	3277.2	3992.0	138.1 ± 3.7	153.4 ± 4.4
10,000	4578.1	4554.8	204.6 ± 10.9	196.0 ± 30.7
12,000	5664.9	5578.1	237.0 ± 23.0	240.8 ± 10.9
14,000	6425.9	5862.2	257.7 ± 6.9	304.7 ± 41.4

Values are the mean ± SE of four mice, except at 10,000 and 14,000 ppm values are the mean ± SE of two mice.
AUC is the area under the curve that describes the concentration of TRI in blood and brain from 0-30 minutes.
C_{max} is the maximum concentration of TRI in blood and brain observed during the 30-minute exposure period.

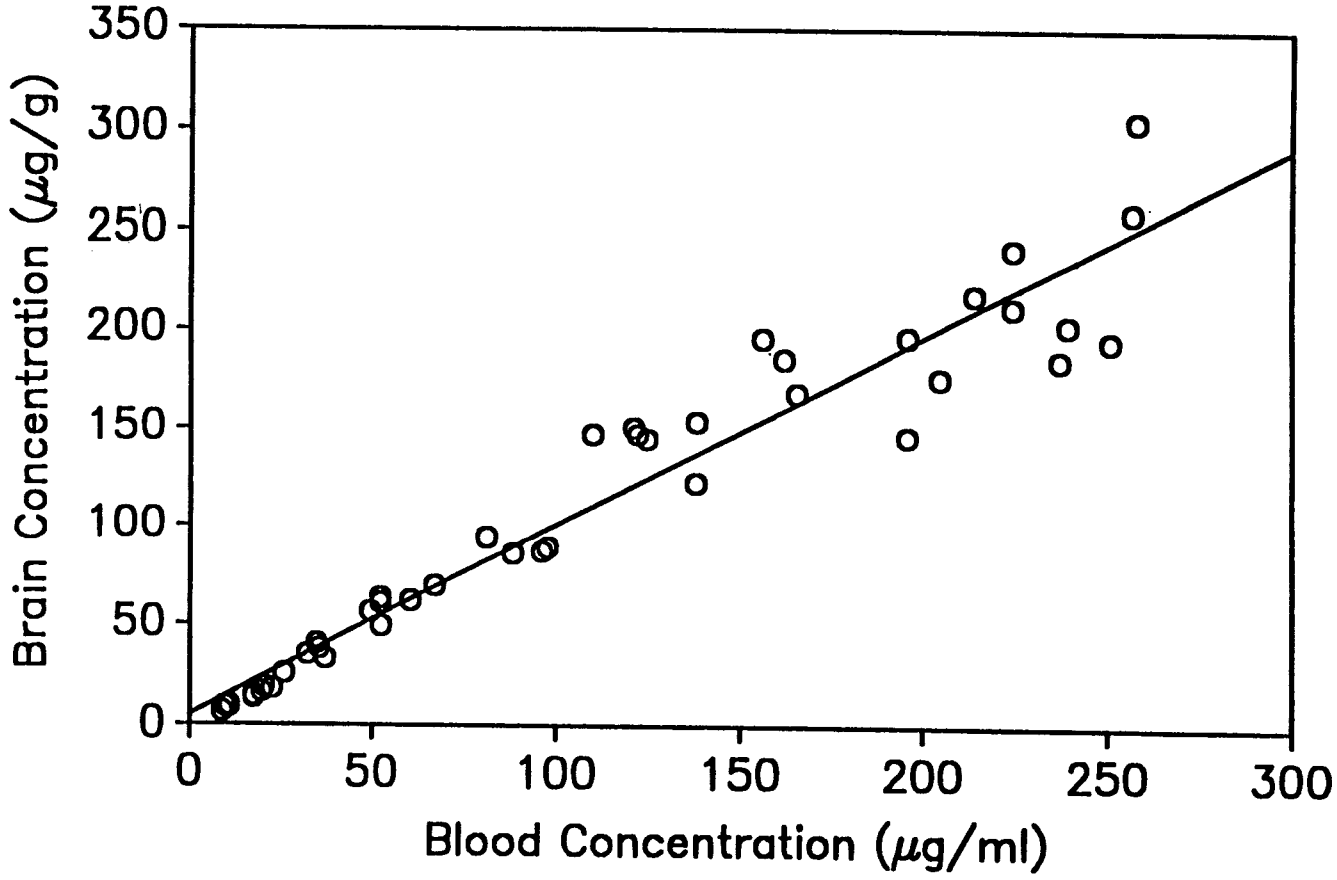

Figure 2: Scatter plot relating blood and brain concentrations of TRI. Each data point represents the mean blood and brain concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to one of nine TRI concentrations. The equation of the regression line is: $y = 0.954x + 5.095$.

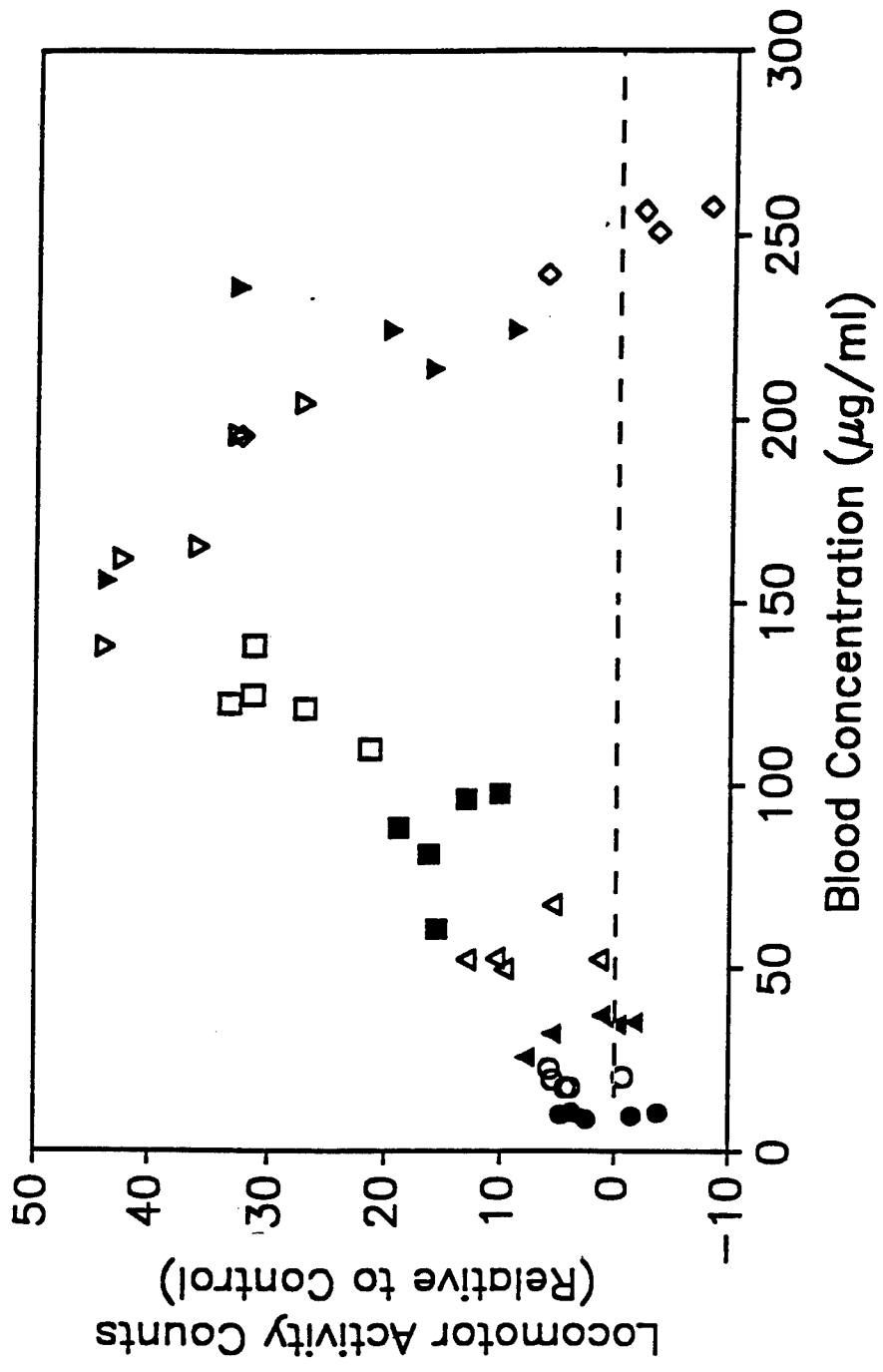

Figure 3: Scatter plot relating the blood concentration of TRI to locomotor activity. Each data point represents the mean blood concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to 500 (●), 1000 (○), 2000 (▲), 4000 (△), 6000 (■), 8000 (□), 10,000 (▼), 12,000 (▽) or 14,000 ppm TRI (◇), as well as the mean locomotor activity of ten mice at corresponding times and exposure concentrations.

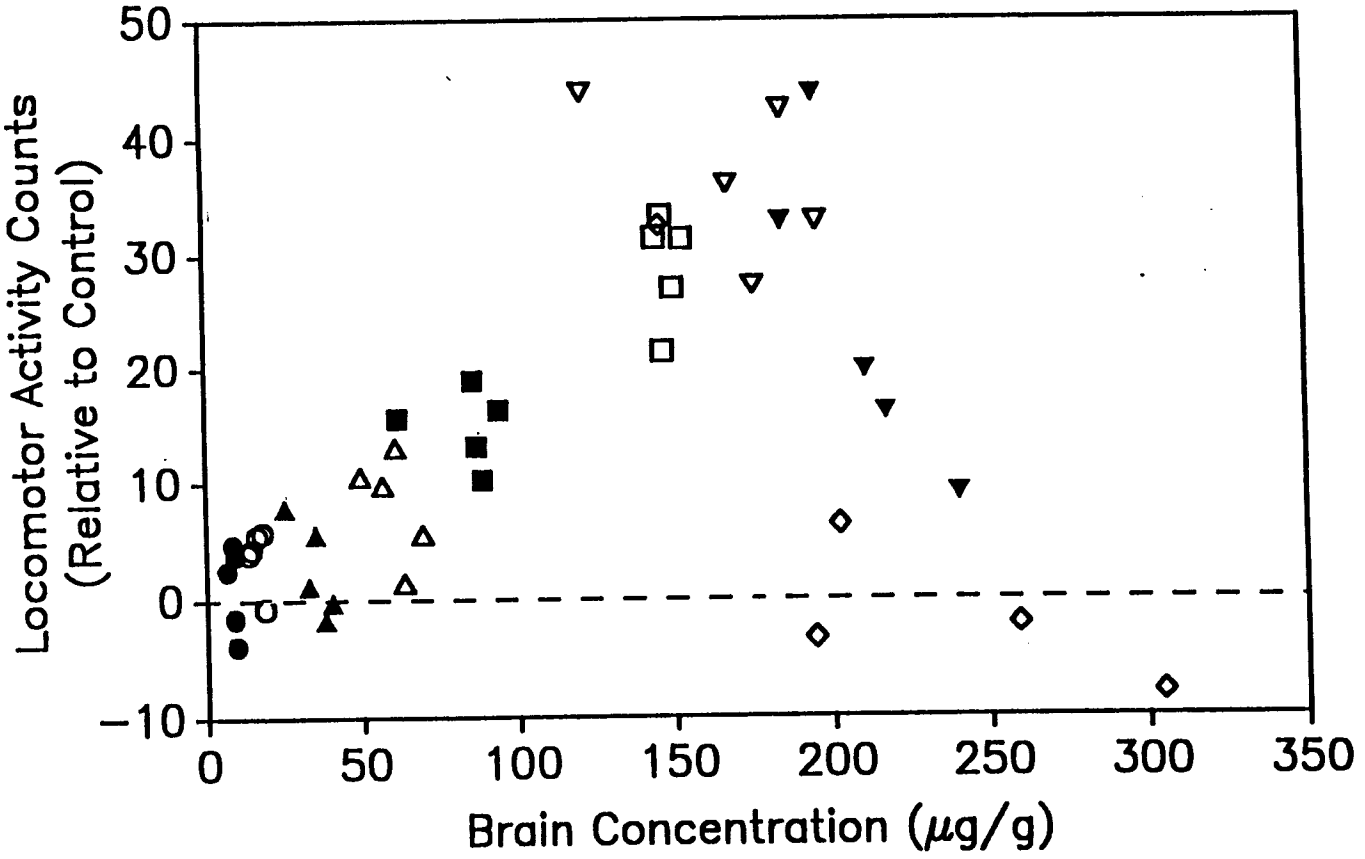

Figure 4: Scatter plot relating the brain concentration of TRI to locomotor activity. Each data point represents the mean brain concentration of two (12,000 and 14,000 ppm) or four mice after 6, 12, 18, 24 or 30 minutes of exposure to 500 (●), 1000 (○), 2000 (▲), 4000 (△), 6000 (■), 8000 (□), 10,000 (▽), 12,000 (▼) or 14,000 ppm TRI (◇), as well as the mean locomotor activity of ten mice at corresponding times and exposure concentrations.

SUMMARY AND CONCLUSIONS

Physiological and anatomical differences between species can influence the pharmacokinetics of halocarbons considerably. As a result, equivalent exposures to different species may not result in equivalent doses to target tissues, including the CNS. Therefore, the extrapolation to humans of neurobehavioral toxicity data collected in experimental animals, in the absence of comparative pharmacokinetic analysis, can lead to erroneous conclusions. However, if the assumption is true that halocarbon concentrations in the CNS of one species are equally as toxic in another, the behavioral response of humans to various halocarbon exposures might be predicted from animal data where brain dose is correlated with changes in behavior. Of potential use in this approach to interspecies extrapolation are physiologically-based pharmacokinetic (PBPK) models that can accurately predict tissue halocarbon concentrations in humans over time, under a variety of exposure conditions. At present, interspecies extrapolations of neurobehavioral toxicity data on the basis of brain dose are virtually non-existent, due to the paucity of data on halocarbon tissue kinetics, neurobehavioral toxicity, and their quantitative relationship.

Using highly quantitative behavioral measures and a sensitive analytical method, time-courses of blood and brain concentrations and neurobehavioral toxicity were determined for two halocarbon solvents in two rodent species. Based on these data, quantitative relationships, or the lack thereof, have been reported between the degree of neurobehavioral toxicity and internal measures of dose. In the case of orally-administered PCE, relationships between blood and brain concentrations and operant

performance were not discernable, due in part to an acute adaptation of rats to PCE's response suppressing effect. For inhaled TRI, blood and brain concentrations were strongly correlated with the rate of operant responding in rats. Responding was slightly in excess of control rates at low concentrations, and decreased in a linear fashion as blood and brain concentrations increased. A robust biphasic response was seen in the locomotor activity of mice exposed to TRI by inhalation. Locomotor activity increased monophasically as solvent concentrations increased to a threshold concentration, above which activity declined and eventually fell below the control level.

Considering the hypothesized mechanism by which solvents affect the CNS, brain concentration would appear to be a logical dose metric to correlate with behavioral toxicity. Our studies with TRI indicate that blood concentrations are highly correlated with brain concentrations, and thus would also be suitable for such a purpose. This is important since it suggests that blood levels in humans may be used to predict brain levels, and thus the degree of behavioral impairment. There appear to be important limitations, however, to the use of blood and brain concentrations as dose metrics. For example, it can be argued that the differential distribution of solvents in the brain makes it inappropriate to consider the CNS as a single homogeneous compartment for kinetic purposes or as a dose metric. Moreover, that behavioral changes in SCOB and locomotor activity may be mediated by a solvent effect other than that exerted on the CNS cannot be discounted. Systematic quantitative relationships between blood and brain solvent concentrations and the accompanying behavioral changes are also hindered by such confounders as acute neuronal adaptation, as was demonstrated in the study of PCE; biphasic response patterns, as was demonstrated for TRI on locomotor activity; and the apparent role of solvent uptake rate, as was evident in TRI's effect on operant responding.

In addition to specific concerns regarding dose metrics, solvent-induced human psychomotor impairment appears to occur at lower blood concentrations than do

behavioral effects in animals. This suggests that human psychomotor tests are more sensitive to solvents than behavioral tests employing animals. It is therefore incumbent upon practitioners of behavioral toxicology to devise tests in animals that measure some of the same behaviors that have proven so sensitive in humans. As was demonstrated by comparing dose-response curves for TRI's effect on the operant behavior of singly and repeatedly exposed rats, it is also important to have a thorough knowledge of factors that influence behavior in order to correctly interpret behavioral dose-response data and its suitability for extrapolation to humans.

There is a clear need for an alternative to the current practice of setting exposure limits by applying safety factors to animal data to account for uncertainties inherent in extrapolation on the basis of exposure concentration. The blood and brain concentration time-course data in this dissertation are currently of value for the validation and refinement of PBPK models for animals. The behavioral data sets in this dissertation are important additions to the toxicology literature in that they include the first studies of PCE's effect on operant responding and TRI's effect on the operant responding of rats, and a vast expansion of the concentration range used in the one existing study of TRI's effect on locomotor activity. However, the behavioral and pharmacokinetic data will be particularly valuable when used together to validate PBPK models that can predict the tissue pharmacokinetic patterns of halocarbons in experimental animals, and thus the magnitude of neurobehavioral toxicity expected. Validation of a PBPK model using this data could be very useful once the model is scaled up to humans, allowing insight into what exposure levels may or may not produce behavioral effects in humans. To simply apply biochemical and pathological measures of neurotoxicity to PBPK models, in lieu of behavioral measures, would be shortsighted. After all, it can be argued that neurological effects with no functional or behavioral consequences are of little concern, while significant behavioral deficits without currently recognizable neurological bases are still significant deficits. It is certain that in the coming years, there will be exciting

opportunities to use pharmacokinetic data to impart a more scientific basis to the assessment of human risk for neurobehavioral toxicity.